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Name of Candidate: Sarah Shafer Berger
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DISSERTATION AND ABSTRACT APPROVED:

DATE:

Tracy Sbrocco, Ph.D. 8/26/09
Department of Medical & Clinical Psychology
Committee Chairperson

Neil Grunberg, Ph.D. 8/26/09
Department of Medical & Clinical Psychology
Dissertation Advisor

Marian Tanofsky-Kraff, Ph.D. 8/26/09
Department of Medical & Clinical Psychology
Committee Member

Ildy Katona, M.D. 8/26/09
Department of Medical & Clinical Psychology
Committee Member

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Stress has negative effects on mental health (e.g., anxiety and depression) and physical health (e.g., cardiovascular diseases) and social support can attenuate the harmful effects of stress. It is not clear the extent to which stress during sensitive periods of life, for example during the prenatal period, can increase subsequent risk factors for mental and physical health. It also is not clear if social interventions during the prenatal period can attenuate any long-term detrimental consequences of stress.

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Sarah Shafer Berger

Sarah Shafer Berger
MEDICAL AND CLINICAL PSYCHOLOGY PROGRAM
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ABSTRACT

Title of Thesis: Behavioral and biological effects of prenatal stress and social enrichment: Relevance to heart disease

Author: Sarah Shafer Berger, Doctor of Philosophy, 2009

Thesis directed by: Neil E. Grunberg, Ph.D., Professor
Department of Medical and Clinical Psychology

Stress has negative effects on mental health (e.g., anxiety and depression) and physical health (e.g., cardiovascular diseases) and social support can attenuate the harmful effects of stress. It is not clear the extent to which stress during sensitive periods of life, for example during the prenatal period, can increase subsequent risk factors for mental and physical health. It also is not clear if social interventions during the prenatal period can attenuate any long-term detrimental consequences of stress.

This doctoral research project was designed to examine the effects of prenatal stress with and without social enrichment on physical and mental health risk factors relevant to cardiovascular disease. A rat model was used to determine: (1) the biological and behavioral consequences of chronic prenatal stress relevant to cardiovascular disease; and (2) whether social enrichment intervention can attenuate any detrimental effects of prenatal stress.

This research was a full factorial design with the independent variables of prenatal stress or no stress, prenatal isolation or pair housing (i.e., social enrichment), and male or female offspring. The offspring were the subjects of interest. The dependent variables were biological (body weight, serum corticosterone, blood glucose, insulin, cholesterol, c-reactive protein, heart

morphology) and behavioral (food consumption, open field locomotor activity, elevated plus maze, forced swim test, and social interaction) variables relevant to cardiovascular disease risk.

Prenatal stress and early environment had a *long- term* impact on biological and behavioral indices of health. Prenatal stress increased corticosterone levels, increased negative social interactions, and altered heart morphology for both sexes, and lowered body weight, C-Reactive Protein (CRP) and glucose for males only. A prenatal social environment resulted in lower CRP and changes in heart morphology for both sexes and greater insulin, activity, anxiety, depressive-like behavior, and a greater amount of social interaction for males only. Overall, social environment did not attenuate the detrimental effects of prenatal stress. The results revealed that prenatal stress and social environment have physical and mental effects that last well into adulthood in rats and, as a result, may impact cardiovascular disease risk.

BEHAVIORAL AND BIOLOGICAL EFFECTS OF PRENATAL STRESS AND SOCIAL ENRICHMENT: RELEVANCE TO HEART DISEASE

by

Sarah Shafer Berger

Doctoral Dissertation submitted to the Faculty of the
Department of Medical and Clinical Psychology
Graduate Program of the Uniformed Services University
of the Health Sciences in partial fulfillment
of the requirements for the degree of
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friendship. One of the many blessings of my marriage is my extended family. My in-laws, including the Berger and Robbins extended families, have embraced me and my professional endeavors.

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INTRODUCTION

Overview

It is well documented that stress has negative effects on mental health (e.g., increased anxiety and depression) (Dozier & Peloso, 2006; Heim & Nemeroff, 2001) and physical health (e.g., increased cardiovascular diseases) (e.g., Selye, 1956). It also has been reported that social support in humans (Bauer, Perks, Lightman, & Shanks, 2001; Bisson, Brayne, Ochberg, & Everly, 2007; Flaherty, Gaviria, Black, Altman, & Mitchell, 1983; Houston, Cooper, & Ford, 2002; Cohen & Wills, 1985) and social enrichment in animals (Diamond, 1967; Rosenzweig & Bennet, 1996; Shafer, 2006; Tomchesson, 2005) can improve mental and physical health and attenuate stress effects. Social support in humans appears to be particularly important in cardiovascular disease risk (e.g., Kop et al., 2005). Animal studies of social enrichment report potentially beneficial effects in the behavioral reactions to drugs of addiction (Green, Cain, Thompson, & Bardo, 2003; Green, Gehrke, & Bardo, 2002), changes in feeding and body weight (Shafer, 2006; Tomchesson, 2005), and recovery from brain injury (Elliott, Faraday, Phillips, & Grunberg, 2004).

It is not clear the extent to which stress during sensitive periods of life, for example during the prenatal period, can increase subsequent risk factors for mental and physical health. It also is not clear if social interventions (e.g., social enrichment) during the prenatal period can attenuate any detrimental consequences of stress during these periods of life. If social enrichment can attenuate the **long-term** effects of prenatal stress, then social enrichment could

have a robust clinical impact. If social enrichment attenuates subsequent health problems, then social enrichment could provide a valuable intervention to change the developmental trajectory for children who might otherwise suffer extreme predispositions to later mental and physical health problems.

This doctoral research project examined effects of prenatal stress with and without social enrichment on indices of mental and physical health in rats from young ages through adulthood. Behavioral and biological measures relevant to cardiovascular diseases were the focus of this project because cardiovascular diseases are the leading cause of death and illness (American Heart Association, 2007). Also, there are well-established relationships between cardiovascular diseases and psychological/behavioral variables (including stress, body weight, anxiety, depression, and social support). The research design involved the use of an animal (rat) model of prenatal stress thereby allowing for manipulation of stress and housing to assess: (1) causation; (2) evaluation of subjects from birth into early adulthood; (3) detailed behavioral measures; and (4) collection of biological measures (e.g., corticosterone, C-Reactive Protein, heart morphology). The use of a rat model allowed for the study (from prenatal exposures all the way to adulthood) to be completed in less than a year, rather than the decades it would take in humans. It also allowed for 100% participation every day for four months. Longitudinal assessments with frequent measures and high subject retention are extremely difficult to conduct with human participants.

The long-term consequences of prenatal stress and social enrichment intervention to attenuate the consequences of prenatal stress were examined

using a 2 x 2 x 2 full factorial design. The independent variables were stress (or no stress) during pregnancy, isolation or pair housing (i.e., social enrichment) of the dam during pregnancy, and male or female offspring. The offspring were the subjects of interest. The dependent variables were biological (body weight, corticosterone, cholesterol, C-Reactive Protein, serum glucose, insulin, heart morphology) and behavioral (food consumption, open field locomotor activity, elevated plus maze [to index anxiety], forced swim test [to index depression] and social interaction) variables relevant to cardiovascular diseases.

The specific aims of the proposed research were to determine: (1) the biological and behavioral consequences of chronic prenatal stress relevant to cardiovascular disease; and (2) whether prenatal social enrichment attenuates the detrimental effects of prenatal stress.

This paper first reviews the literature on stress and social support. Next, the rationale for each independent and dependent variable included in this research project is provided. Then, preliminary research studies are presented, followed by the methods of this project. Finally, the results and a discussion of the findings are presented.

Stress

"Stress is the process by which environmental demands (i.e., stressors) tax or exceed the adaptive capacity of an organism, resulting in psychological and biological changes that may place a person at risk for disease" (Cohen et al., 1995, p. 3). This psychobiological definition of stress considers several different aspects of stress and the stress response as well as its effects on health.

Historical Context of Stress

Early conceptualizations of the stress response focused on biology. Walter B. Cannon (1935) suggested that organisms respond to events or challenges to an internal homeostasis with reactions that attempt to restore a balance within the body. Cannon indicated that illness results when an organism is chronically activated to maintain homeostasis in response to an imbalance caused by environmental events. Hans Selye (1973) also conceptualized the stress response from a biological perspective. According to Selye's (1973) General Adaptation Syndrome (GAS), "stress is a non-specific response of the body to demands for adaptation," primarily involving the Hypothalamic-Pituitary-Adrenal (HPA) Axis (Selye, 1973, p. 32). Specific events, positive or negative, activate the HPA axis, resulting in various biological responses.

Later stress theorists emphasized the mind-body interaction with regard to stress. John Mason (1975) suggested that the individual's experience of stress depends on one's appraisal of a situation or stimulus, personality factors, situation or environmental influences, and an integrated multi-hormonal response. Rahe and Arthur (1978) attempted to quantify stress-inducing events by examining an individual's level of stressful experiences. Richard Lazarus and colleagues emphasized the contribution of cognitive factors in the individual's response to a stressor (Lazarus, 1966; Lazarus & Folkman, 1984). Other investigators emphasized the role of perceived controllability and predictability (i.e., cognitive control) in determining a person's response to stress (Glass & Singer, 1972).

Bruce McEwen (1998) conceptualized stress as an integration of biological and psychological factors. McEwen, like Cannon and other stress researchers, emphasized that stress in the short-term is protective, but that it is potentially damaging in the long-term (McEwen, 1998). McEwen termed the short-term, protective effect of stress "allostasis," meaning change through stability, and the long-term detrimental consequences are termed "allostatic load." The allostatic load refers to cumulative effects over time that affect health either directly (e.g., increased blood pressure) or indirectly through behaviors (e.g., cigarette smoking which increases the risk of disease). Genes, early development, isolation, and life-style behaviors can all affect allostatic load (McEwen, 1998).

Biological, psychological, and environmental variables all are critical to stress responses. Therefore, it is important that stress research use an interdisciplinary approach that considers all of these factors. The present research was designed to examine stress with consideration of environmental factors and responses that involve both biological and psychological reactions.

The Heath Effects of Stress

The 2006 Gallop Poll indicated that 3 out of 4 Americans report that they "sometimes" experience stress in their daily life and that 4 out of 10 Americans report experiencing stress "frequently" in their daily life (Carroll, 2007). Women report marginally higher stress levels than men report (40% vs. 35%) (Carroll, 2007). These statistics are alarming because chronic stress can lead to negative health consequences. Physically, chronic stress can lead to heart disease,

immune-mediated conditions, or other health conditions (e.g., Markovitz & Matthews, 1991; Kiecolt-Glaser, Marucha, Malarkey, Mercado, & Glaser, 1995). It is estimated that 75 - 90% of visits to physicians are the result of stress (e.g., symptoms of extreme pain, fatigue, high blood pressure) (American Institute of Stress, 1996). Stress also can affect mental health, most commonly by increasing risk for depression or anxiety (Anisman & Zacharko, 1992; Baum, Cohen, & Hall, 1993). Mental health also can affect physical health. For example, depression is now recognized as a strong risk factor for heart disease (e.g., Kop & Gottdiener, 2005). The present experiment focused on cardiovascular disease risk, including direct physical health factors (e.g., body weight, heart morphology) and indirect mental health risk factors (e.g., depression and anxiety).

Stress is a risk factor for many cardiovascular diseases including atherosclerosis, hypertension, stroke, myocardioal infarction, and sudden cardiac death (Krantz, Kop, Santiago, & Gottdiener, 1996). It has been proposed that stress can lead to cardiovascular diseases through its effects on the catecholamines (epinephrine or norepinephrine) via sympathetic nervous system activation, or through the hypothalamic-pituitary-adrenal axis (HPA) axis. Stress increases the release of catecholamines from the adrenal glands, and catecholamines directly increase heart rate via adrenergic receptors on the heart. Stress also results in a slower endocrinological response via the HPA axis in which corticotropin releasing factor (CRF) is released from the hypothalamus, which signals release of adrenocorticotrophin hormone (ACTH) from the pituitary

and cortisol (or corticosterone in rats) from the adrenals. Excess levels of cortisol increase blood pressure causing the heart to work harder, putting it at risk for hypertrophy. Heart disease is the leading cause of all death in the United States, accounting for nearly 40% of all deaths (Centers for Disease Control, 2005). Heart disease is expensive, costing the United States approximately $394 billion per year in health care (Centers for Disease Control, 2005).

Stress also influences food consumption and body weight, known behavioral risk factors for cardiovascular disease. The directional effect of stress on food and body weight is not clear. Stress is thought to lead to obesity and there are some reports to support this belief (e.g., Greeno & Wing, 1994). However, there are many reports suggesting that stress decreases food consumption and body weight (e.g., Levine & Morley, 1981). These contradictory findings may be due to individual differences and/or acute versus chronic stressors. In humans, stress decreases eating in men, but it may increase eating in women, particularly for sweet or bland foods (Grunberg & Straub, 1992). In animals, acute, physical stress (e.g., tail pinch, cold swim) increases eating (Greeno & Wing, 1994). However, psychological stress in rodents (e.g., restraint stress or predator stress) may produce different results. For example, Shafer (2006) reported that rats exposed to restraint stress ate less and weighed less than rats not exposed to restraint stress. Although stress affects eating, the direction of this relationship appears to depend on several variables (e.g., type of stressor, individual differences, food type).

Stress also can have negative mental health effects (Anisman & Zacharko, 1992; Baum, Cohen, & Hall, 1993). Mental health consequences of stress include anxiety, depression, substance abuse, eating disorders, and suicide (Anisman & Zacharko, 1992; Baum et al., 1993). The two most prevalent mental health conditions are depression and anxiety. Approximately 5-20% of the population suffers major, incapacitating depression causing hospitalization and/or a significant period of work absence (Robins et al., 1984) and at least 16% of the population suffers from anxiety (Kessler, McGonagle, Swartz, Blazer, & Nelson, 1993; Liebowitz, 1997). Similar to depression, anxiety can start at a young age and lead to periods of disability (Liebowitz, 1997; Kessler, 1993). It has been hypothesized that stress can lead to depression or anxiety through glucocorticoids (e.g., cortisol or corticosterone) and/or over-activation of the sympathetic nervous system. Glucocorticoid levels are usually abnormal in individuals who are depressed, perhaps because of a disruption in the HPA feedback system. In other words, the body is producing excess cortisol (or corticosterone in rats) and the brain is not receiving the message to stop activating the HPA axis.

There is increasing concern that mental health, in turn, can affect physical health. Depression is a strong risk factor for cardiovascular disease (Kop, 1997; Rozanski, Blumenthal, & Kaplan, 1999). Pratt et al. (1996) reported that individuals with a history of depression were four times more likely to have a heart attack, compared with individuals who did not have a history of depression. The exact mechanism of how depression leads to heart

disease is unknown. Some researchers postulate that depression leads to bad lifestyle choices (e.g., poor diet, cigarette smoking) and other researchers suggest that there are biological reasons for the depression - heart disease comorbidity. Two hypothesized biological mechanisms include: (1) the stress of depression, which may increase cortisol production leading to arrhythmias, increased cholesterol, and an accumulation of abdominal fat. Norepinephrine is also increased in the blood which can lead to increased blood pressure; or (2) immune parameters which are altered in depressed individuals. These parameters may include C-Reactive Protein (CRP), fibrinogen, interleukin-6 (IL6), and adhesion cellular molecule -1 (ICAM-1). Depression can increase IL6 through hormones that cause IL6 to be released from adipose tissue (Empana et al., 2006).

Stress may increase risk of heart disease through direct physical effects or through indirect effects such as depression. As a result, when examining the role of stress contributing to cardiovascular disease risk, it is important to examine physical and mental health effects of stress. This research investigated how prenatal stress leads to biological (including body weight, corticosterone, glucose, insulin, cholesterol, C-Reactive Protein, and heart morphology) and behavioral consequences relevant to cardiovascular risk (food consumption, measures of activity, indices of anxiety, an index of depression, and social interaction).

The Measurement of Stress

Stress can be measured in the laboratory, clinic, or field. Stress also can be examined in humans or animals. The long-term effects of prenatal stress are more complicated to assess, but also can be measured in different ways. Prospective, longitudinal studies with human participants would be a valuable and perhaps ideal approach because these types of studies have the best external validity. However, human longitudinal studies examining effects of prenatal stress on long-term (i.e., into early adult) physical and mental health relevant to cardiovascular disease present several problems: (1) it is unethical to manipulate stress in fetuses for research purposes; (2) such an experiment would take decades to collect data and assess results; (3) there would be many intervening variables and potential confounding variables; and (4) the financial costs of such an experiment would be extremely high.

Alternatively, an animal model provides a feasible way to examine a potential causal relationship between stress during sensitive periods of life and effects relevant to cardiovascular disease risk. The use of an animal model allows for: (1) the manipulation of stress during the prenatal period; (2) control of subject population (to control for individual differences); (3) control of environmental variables (e.g., housing, food and water access, exposure to stressors); (4) frequent behavioral measures; (5) assessment of biochemical measurements of stress; (6) assessment of heart tissue; and (7) a large amount of data collection in less than a year. Animal models have been used for decades in research examining depression (Overmier & Seligman, 1967), anxiety

(e.g, File, 1987), stress (e.g., Baum, Grunberg, & Singer, 1982; Henry et al., 1971; Weiss, 1968; Winders, Grunberg, Benowitz, & Alvares, 1998) and behavioral medicine (Miller, 1969; Miller & Dicara, 1967; Miller & Dworkin, 1974), including cardiovascular diseases (Henry et al., 1971; Herd, 1978; Miller & Mallov, 1977; Surwit, Shapiro, & Good, 1978).

Stress and Development

Stress can occur during any time in the lifespan: prenatally, early life (e.g., childhood), adolescence, adulthood, or late in life (i.e., during the elderly time period). However, it is unclear if stress during one life period may have more of a lasting impact than stress during another life period. In other words, are there life periods during which the impact of stress may be more deleterious? This idea of "critical" periods was first proposed by ethologists (Lorenz, 1981). A critical period is a specific amount of time in which an organism is biologically ready to attain specific behaviors that will aid in its survival. In order for these behaviors to be acquired, the organism needs the support of an environment that is stimulating and can respond to its needs (Berk, 2001). The field of child development later determined that a "sensitive" period is a more appropriate term to use in the context of development (Bornstein, 1989). A sensitive period is a time during which its ideal for an individual to display specific skills and behaviors and when the individual is particularly responsive to his/her environment (Berk, 2001).

The prenatal period is often considered a sensitive period, in part, because the body and brain are growing rapidly. There is something about

growth during the prenatal period that makes this period of life particularly sensitive to external influences. For example, this period is sensitive to effects of drug exposure. Nicotine exposure during the prenatal period can lead to low birth weight and lasting effects on attention and learning (Fried & Makin, 1987; Tizabi, Russell, Nespor, Perry, & Grunberg, 2000). Stress may be an external influence that exerts a particularly powerful effect during the prenatal time period. In fact, some reports suggest that prenatal stress correlates with detrimental health effects (e.g., heart disease) in adulthood (Barker, 2004; Huizink, Mulder, & Buitelaar, 2004; Louey & Thornburg, 2005). Causation, however, is difficult to determine in human studies that rely on retrospective data. It also is unknown whether these effects are relevant to long-term health. The present research project used an animal model to determine if there is a causal relationship between prenatal stress and adult health.

Summary of Stress Relevant to Proposed Work

In summary, stress is a psychobiological process that can negatively impact health relevant to cardiovascular disease risk. It is unclear if there are sensitive periods during the lifespan in which stress exposure may have particularly powerful long-term effects on heart disease risk. The present research used an animal model to examine long-term consequences of prenatal stress on behavioral and biological risk factors of cardiovascular diseases.

Social Support and Social Enrichment

Social support

Social support is a potent mediator of stress and is defined as a feeling that a person is cared about and valued by other people and that he or she belongs to a social network (Baum & Posluszny, 1999). However, it is unclear how social support mediates stress. It may be that perceived social support is necessary (Cohen & Wills, 1985). In other words, a person may have to perceive that he or she has social support for the stress-mediating effect to occur. There are other hypotheses that emphasize the type of support rather than the perception of social support (Wills, 1985). Various types of social support have been defined: (1) esteem support is an interaction with others that increases feeling of self-esteem; (2) information support involves getting necessary information from others; and (3) instrumental support refers to the physical assistance one gets from another (e.g., a ride to the grocery store).

Other researchers have hypothesized that the benefits of social support are based on the mere social presence of another. Triplett (1897) reported that bike racing performance increases when there is just the bodily presence of another and coined the term "social facilitation." Dashiell (1930) reported that the increased performance depended on whether the social presence was a competitor or just watching. Pessin (1930) noted that memorization (a cognitive rather than a physical measure) was improved in certain social situations. Zajonc and colleagues argued that the effects of the social environment to improve or to harm cognitive performance depended on how well an individual

knew the material and that an individual's dominant response increased in the mere presence of others because these situations are arousing (Zajonc & Sales, 1966). Bond and Titus (1983) also reported that mere presence of others affects physical and cognitive performances.

Whether social support requires particular perceptions and emotions is not clear. Social support certainly requires social presence (or perception of social presence). A parallel construct appears in animal research - social *enrichment* - in which animals are housed in pairs or groups to provide opportunities for social interaction. Enrichment (also called environmental enrichment) has been around for over 100 years and has powerful effects.

Historical Context of Enriched Environments

Charles Darwin (1874) reported that brains of domestic rabbits were considerably smaller compared to the brains of wild rabbits. He argued that the reduced brain size of the domestic animals was a consequence of a deprived environment because domesticated animals did not exert their intellects, instincts, or senses as much as animals did in the wild.

Donald Hebb (1947) reported that laboratory rats that he had taken home for his children to play with exhibited superior performance on maze learning when taken back to the laboratory compared to rats that had never left the laboratory environment. Hebb concluded that nerve cells in the brains of the rats had changed in response to the enriched and varied experiences outside the laboratory. He hypothesized that the number of synaptic connections increased and that these structural changes resulted in functional (i.e., behavioral)

modifications. Hebb believed that these changes ("neuroplasticity") reflected new learning. This particular report of Hebb was consistent with Darwin's (1874) observation.

Psychologist Mark Rosenzweig (1966) introduced what became the classic paradigm for studying the impact of enriched environments on rats. Rosenzweig and a neuroanatomist colleague, Marian Diamond, also analyzed the brains of enriched and isolated rats. They reported that enriched rats had heavier cortices, greater capillary diameters in the cortex, and more acetylcholinesterase activity compared with isolated rats (Diamond, 1967).

In enrichment paradigms, animals are housed in groups to provide opportunities for social interaction (i.e., social enrichment). Physical stimulation (i.e., physical enrichment) involves providing objects in the cages to allow tactile stimulation and physical activity (Rosenzweig, 1966; Woodcock & Richardson, 2000). Most environmental enrichment studies (Mohammed et al., 1993; Pham, Soderstrom, Winblad, & Mohammed, 1999) have included social and physical enrichment components. Enriched environments are distinguished from non-enriched environments by the amount of stimulation, activity available, and opportunities for social interaction that are available in the environment. The standard non-enriched environment limits the physical and social enrichment by housing the animals individually without objects (Varty, Paulus, Braff, & Geyer, 2000). Commonly, across human and animal research, environmental enrichment refers to physical and social stimulation provided in the environment.

Interestingly, more recent research in enrichment has indicated differential effects between physical and social enrichment. Elliott and Grunberg (2005) reported that social enrichment has the most profound results as it improves cognitive performance (i.e., increasing simple learning in an open field) for both males and females. Pietropaolo and colleagues (2004) reported that physical and social enrichment effects are different and not additive. Social enrichment leads to decreased activity in an open-field (i.e., improved simple learning) and more affiliative and less aggressive behavior in social situations. Moreover, socially-enriched animals showed biochemical changes including higher levels of brain-derived growth factor (Pietropaolo et al., 2004), but physically enriched animals did not. This research suggests that it is important to differentiate between physical and social enrichment in research. In particular, social enrichment may have some added benefits with regard to health outcomes.

Health Effects of Social Support and Enrichment

As previously stated, social support can attenuate stress responses. With regard to the present research project, it is relevant that social environment affects cardiovascular function and cardiovascular disease risk factors (Kop et al., 2005). High levels of social support (in humans) are associated with lower heart rate, lower blood pressure, lower catecholamine levels, and stronger immune function (e.g., Cohen & Wills, 1985). In contrast, low social support is correlated with a 1.5 to 3 - fold increased risk of future cardiac disease and mortality (Berkman, 1982; Woloshin et al., 1997).

Social support also has been reported to be beneficial for mental health. Social support expedites treatment for depression (Flaherty et al., 1983; Houston et al., 2002), anxiety disorders, and posttraumatic stress disorder (Bisson et al., 2007).

Social enrichment in animals also has produced changes in indices of physical and mental health. Elliott and Grunberg (2005) reported that social enrichment resulted in faster recovery from brain injury compared with animals housed in isolation. Other researchers have reported that social enrichment decreases food consumption and body weight to levels that are still in a healthy range (Shafer, 2006; Tomchesson, 2005). Shafer (2006) also reported that social enrichment, in particular, attenuates effects of stress on heart morphology. Specifically, the hearts of animals exposed to stress had altered dimensions on the septal wall, heart length, and the right and left ventricles, yet the hearts of animals exposed to stress and enrichment had less alteration in the heart's dimensions. In other words, it appeared that enrichment attenuated the effect of stress on the heart's structure.

In addition to these effects of enrichment on physical health, there are effects of social enrichment on measures relevant to mental health. Enrichment reduced the effect of freezing behavior (an index of anxiety) in early-life stressed rats (Imanaka, Morinobu, Toki, & Yamawaki, 2006). Mice exposed to enrichment also showed less anxiety (as assessed by greater time in open arms of the elevated plus maze) compared with isolated mice. Enrichment also decreased

the effects of stress on freezing behavior and corticosterone levels in mice (Benaroya-Milshtein et al., 2004).

Summary of Social Support Relevant to the Proposed Work

Stress may increase the risk of heart disease through direct physical effects or through psychobiological effects. Therefore, when examining the role of stress in contributing to cardiovascular disease risk, it is important to examine both physical and mental health consequences of stress. It also is important to determine if stress during sensitive developmental periods, such as the prenatal period, can be attenuated by social enrichment. The present experiment examined effects of prenatal stress on biological consequences (including body weight, corticosterone, glucose, insulin, cholesterol, C-Reactive Protein, and heart morphology) and behavioral consequences relevant to cardiovascular risk (food consumption, measures of activity, indices of anxiety, an index of depression, and social interaction). The present study also examined if social enrichment for the dam during the prenatal period attenuates effects of stress on the long-term health of the offspring.

Stress and Social Enrichment in a Biobehavioral Animal Model

This section reviews research relevant to each independent and dependent variable in the present research. In the current project, pregnant dams were exposed or not to a predator and unpredictable stress (see Table 2) and were housed either in isolation or in social enrichment. The male and female offspring were the subjects of interest and the dependent measures were

biological and behavioral variables relevant to cardiovascular health. The biological dependent variables were body weight, corticosterone, serum glucose, insulin, cholesterol, C-Reactive Protein, and heart morphology. The behavioral dependent variables were food consumption, locomotor activity, center time (to model anxiety), elevated plus maze (to model anxiety), swim test (to model depression), and social interaction.

Independent Variables

Stress. As discussed in Berger and Grunberg (in preparation), Perry (2009), and other researchers, predator stress is an effective stressor that is implemented using real or synthetic odors of a natural predator (e.g., fox). Exposure to predator stress reliably produces increases in stress hormones (Berger & Grunberg, in preparation; Campbell, Lin, DeVries, & Lambert, 2003; Hayley, Borowski, Merali, & Anisman, 2001; Perry, 2009). Exposure to predator stress also produces behavioral changes in rodents including differences in food consumption, elevated plus maze, startle response, freezing behavior, withdrawal behavior, and exploratory behavior (Adamec, Head, Blundell, Burton, & Berton, 2006; Belzung, El Hage, Moindrot, & Griebel, 2001; Endres, Apfelbach, & Fendt, 2005; Masini, Sauer, & Campeau, 2005; Mechiel Korte & De Boer, 2003; Takahashi, Nakashima, Hong, & Watanabe, 2005). In the present experiment, predator stress was combined with an unpredictable stressor. Predator stress was presented simply by introducing a piece of cotton with commercially available synthetic fox urine into a test cage with the rat subject. Unpredictable, non-painful stressors included noise, light, and cage shaking.

Unpredictable stress was included because it reliably produces alterations in stress hormones (Fride, Dan, Feldon, Halevy, & Weinstock, 1986; Weinstock, Matlina, Maor, Rosen, & McEwen, 1992) and behavior (Fride et al., 1986; Gonzalez Jatuff, Berastegui, Rodriguez, & Rodriguez Echandia, 1999) in rodents.

Social enrichment. Social enrichment is a housing manipulation that can involve two rats per cage or many rats per cage. Social enrichment has resulted in many behavioral effects. There are mixed reports as to whether it can attenuate the effects of stress (as assessed biologically and behaviorally) (Belz, Kennell, Czambel, Rubin, & Rhodes, 2003; Francis, Diorio, Plotsky, & Meaney, 2002; Morley-Fletcher et al., 2003; Schrijver, Bahr, Weiss, & Wurbel, 2002). The present research housed two dams per cage or one dam per cage during gestation to manipulate social enrichment.

Sex. Rats (i.e., offspring) of both sexes were included for two main reasons: (1) there have been some reports of differential effects of prenatal stress on males compared with females (Frye & Wawrzycki, 2003; Louvart, Maccari, & Darnaudery, 2005; Ordyan & Pivina, 2004). As a result, it cannot be assumed that the biological or behavioral reactions would be the same between the sexes, and (2) using both sexes allows for better modeling of the human population. Examples of sex differences that have been reported include more learning deficits and changes in the prefrontal cortex in prenatally-stressed male offspring (Weinstock, 2007). There also have been reports of increased anxiety, depression, and a decreased response in the HPA axis in response to prenatal stress in female offspring compared with male offspring (Weinstock, 2007).

Female offspring exposed to prenatal stress also have altered cardiovascular responses, including elevations in systolic arterial pressure, alterations in blood pressure, and delayed heart rate recovery after a stressor compared with male offspring (Igosheva, Klimova, Anishchenko, & Glover, 2004). Because of these reports, it cannot be assumed that the biological or behavioral reactions would be the same between the sexes.

Dependent variables.

Each dependent variable is defined and the rationale for its inclusion is provided. Then, the relevant stress literature with regard to that variable is presented (including relevant preliminary study findings).

Body weight and food consumption. Body weight and food consumption are general indices of animal health and growth. These variables were included in the present experiment because body weight and eating are affected by stress (Faraday, 2002) and social enrichment (Long, Shafer, Oates, Marwein, & Grunberg, 2007; Shafer, 2006; Tomchesson, 2005) and because obesity is a known risk factor for heart disease. Varying kinds of stress also produce changes in body weight and eating. For example, prenatal stress during mid to late pregnancy in stress-sensitive dams resulted in male mice offspring being 15% heavier as adults (Mueller & Bale, 2006).

Cortiocosterone. Corticosterone (analogous to cortisol in humans) is released by the adrenal cortex in response to ACTH stimulation. Increases in corticosterone are widely used to index stress responses in rats (e.g., Faraday et al., 2005; Kalinichev et al., 2002; Kant et al., 1987; Hayley et al., 2001).

Corticosterone responses to stressors are considered adaptive and reflect healthy adjustment in the short-term. If corticosterone release is prolonged, then it is considered maladaptive because it can lead to wide-spread physical and mental health problems (e.g., McEwen, 1998).

Stress produces reliable changes in corticosterone. Restraint stress in rodents results in elevated stress hormones including serum corticosterone (Acri, 1994; Kant, Leu, Andersen, & Mougey, 1987; Raygada, Shaham, Nespor, Kant, & Grunberg, 1992). Additionally, increased serum corticosterone levels were found after 14 days of restraint stress for 20 minutes (Faraday, Blakeman, & Grunberg, 2005). Specifically, nonstressed rats had serum corticosterone concentrations at 215 $\pm$ 10 ng/ml and stressed rats had mean serum corticosterone concentrations at 585 $\pm$ 20 ng/ml (Faraday et al., 2005). Corticosterone was used in the present experiments as an index of HPA stress activation.

Cholesterol. Cholesterol is a lipid in the cells of all tissues and is carried in the blood. Abnormally high cholesterol levels and abnormal proportions of low-density lipoprotein (LDL) and high-density lipoprotein (HDL) are correlated with cardiovascular disease risk because they can lead to atherosclerosis (American Heart Association, 2007).

To date, no research has examined the effects of prenatal stress on cholesterol levels in the offspring. Despite the lack of research, this is an important dependent variable to assess cardiovascular disease risk. Moreover, elevations in cholesterol are associated with chronically elevated plasma cortisol

(the human equivalent of corticosterone) in humans (Sapolsky et al., 2000). In other words, chronic stress may affect cholesterol levels. The present research examined if prenatal stress causes changes in cholesterol levels in male and female offspring when they grow into adulthood.

C-Reactive Protein. C-Reactive Protein (CRP) is a marker of inflammation relevant to physical and mental health and is a predictor of cardiovascular events (Ranjit et al., 2007; Ridker, Hennekens, Buring, & Rifai, 2000). Local and systemic inflammation are thought to lead to the initiation and progression of atherosclerosis. C-Reactive Protein and cholesterol levels together are predictors of myocardial infarction and high levels of CRP can indicate poor prognosis in individuals with previous myocardial infarctions (Wong, Black, & Gardin, 2000). C-Reactive Protein also is affected by stress and depression (De Berardis et al., 2006; Dressler, Balieiro, Ribeiro, & Dos-Santos, 2006; Taylor et al., 2006; Toker, Shirom, Shapira, Berliner, & Melamed, 2005).

To date, no research has examined the effects of *prenatal* stress on offspring levels of CRP. There has been a small body of research (in humans) that has examined the effects of *early life* stress on C-Reactive Protein in adulthood. Early life stress in children (e.g., child maltreatment or low socioeconomic status) is significantly correlated with higher CRP levels in adulthood (Danese, Pariante, Caspi, Taylor, & Poulton, 2007; Taylor, Lehman, Kiefe, & Seeman, 2006). The present research examined if prenatal stress *causes* elevations in CRP in young adulthood.

Serum glucose. Serum glucose is the assessment of glucose (sugar from carbohydrates) in the blood and can be obtained after a period of fasting or after an individual has eaten. It was included in the present experiment because higher serum glucose levels can increase risk for heart disease even if an individual does not have diabetes (Kanaya, Grady, & Barrett-Connor, 2002).

Human and animal research findings indicate that prenatal stress is correlated with high glucose in the offspring. This result seems consistent especially when the maternal stressor is malnutrition (e.g., Barker, 2002; Seckl & Holmes, 2007). When the maternal stressor is psychological, the results are not as clear. In the animal literature, prenatal stress increases serum glucose levels (Vallee, Mayo, Maccari, Le Moal, & Simon, 1996; Lesage, Del-Favero, Leonhardt, Louvart, Maccari, & Vieau et al., 2004). There appear to be sex differences, at least in young rodents, as prenatally stressed, female mice (three weeks of age) were reported to have higher glucose levels compared with controls but this differences did not exist for male mice (Mueller & Bale, 2006). The present research examined if prenatal stress caused elevated glucose levels in male and female offspring in adulthood.

Insulin. Insulin is a hormone produced by the pancreas that allows glucose to enter liver and muscle cells. Insulin resistance is part of a generalized metabolic disorder, in which the body cannot use insulin efficiently (also known as Type 2 diabetes mellitus). Insulin resistance combined with the other risk factors like obesity and elevated lipids increases the risk of cardiovascular disease (American Heart Association [AHA], 2007).

The findings in the literature with regard to prenatal stress and insulin are similar to the reports of prenatal stress and serum glucose. There is evidence from animal and human research that prenatal stress is associated with insulin changes in the offspring. Again, this result seems to be consistent if the maternal stressor is malnutrition (e.g., Barker, 2002; Seckl & Holmes, 2007). When the maternal stressor is psychological, the results appear to differ. In the animal literature it has been reported that immobilization stress during one week of gestation did not produce changes in insulin in adult offspring (postnatal day 60 and 120) (D'mello & Liu, 2006) or when offspring were two years old (Lesage et al., 2004). Only male offspring were examined in both of these studies. The present research examined if unpredictable prenatal stress causes alterations in insulin in male and female offspring as adults.

Heart morphology. Heart morphology is the assessment of the heart's dimensions and gross physical characteristics (e.g., left ventricular size, length, width, weight, etc). Heart morphology was included in the present experiment because changes in the heart structure occur in various cardiovascular conditions. For example, left ventricular hypertrophy, a condition affecting approximately 16% of Whites, 21% of American Indians, and 33 to 43% of African-Americans (Gardin et al., 1995), involves expansion of the left ventricle and is a powerful predictor of mortality and morbidity (Ghali, Liao, & Cooper, 1998).

Rodent heart morphology is affected by stress (Elliott, Faraday, & Grunberg, 2003). Male rats have shorter heart lengths, left ventricle cavity

widths, and thicker septal walls in response to restraint stress. Specifically, non-stressed rats had a mean left ventricle cavity of 4.9 $\pm$ 0.4 mm and a mean septal wall width of 2.4 $\pm$ 0.2 mm and stressed rats had a mean left ventricle cavity of 3.8 $\pm$ 0.5 mm and a mean septal wall width of 3.2 $\pm$ 0.2 mm. However, no significant differences were reported for female rats. Another study reported a significant increase in the heart weight of rats receiving various types of stress (including restraint stress) (Nagaraja & Jeganathan, 1999). Shafer (2006) reported that heart morphology in rats was affected by restraint stress during early adolescence. Shafer (2006) also reported some effects of enrichment on the heart, particularly on the posterior wall. Enrichment appeared to attenuate the effects of stress with the most pronounced effects for the septal wall, heart length, left ventricle, and right ventricle measurements. The present research built upon these recent findings in our laboratory. This project examined the effect of prenatal stress on adult heart dimensions and whether social enrichment attenuated any stress-induced changes in heart morphology.

Locomotor activity. Open field locomotion refers to an animal's behavior when placed in a non-home cage arena. Locomotor activity can be an index of: an animal's general health and activity, a measure of simple learning (e.g., habituation to a novel environment), and time spent in the center of the chamber can be an index of anxiety. Locomotor assessment is widely used in animal experiments. It was included in the present experiment to index general arousal and anxiety.

Stress has been reported to decrease open-field activity in rats (Faraday, 2002; Galea, Wide, & Barr, 2001). After 20 minutes of restraint stress, open-field activity was decreased in male Sprague-Dawley and male Long-Evans rats, but only on the first day of stress. Increased center time has been interpreted as decreased anxiety and decreased center time is interpreted as increased anxiety (Beck & Luine, 2002; Gamallo, Villanua, Trancho, & Fraile, 1986; Lee, Tsai, & Chai, 1986). Variations in the amount of restraint and the type of subjects used to investigate stress responses have provided different results.

Stress during the prenatal period also has been reported to affect activity levels in the open field (Deminiere et al., 1992; Louvart et al., 2005) and these effects appear to differ by sex of subjects (Alonso, Arevalo, Afonso, & Rodriguez, 1991; Ordyan & Pivina, 2004). It is unclear if an intervention for the dams during the prenatal period can attenuate some of the behavioral effects in the offspring. Therefore, center time in the open field chamber was used in the present experiment to determine if a prenatal intervention could attenuate anxiety in the offspring.

Elevated Plus Maze (EPM). Elevated plus maze is an index of anxiety-like behavior in rodent research (Elliott et al., 2004; Hogg, 1996; Kalinichev, Easterling, Plotsky, & Holtzman, 2002; Pellow, Chopin, File, & Briley, 1985). It was included in the present experiment to determine if prenatal stress affects anxiety in the offspring.

Stress increases anxiety-like behaviors in the EPM in rodents (Kalinichev et al., 2002; McIntosh, Anisman, & Merali, 1999; Wigger & Neumann, 1999).

Exposure to predator stress also produces an anxiogenic response on the EPM (Adamec, Walling, & Burton, 2004).

Responses in the elevated plus maze also are affected by stress during the prenatal period. Prenatal stress has been reported to result in less time spent in the open arms (i.e., an anxiogenic response) in male and female offspring, with a more pronounced effect in female offspring (Zagron & Weinstock, 2006). There also appears to be an anxiogenic response when prenatally-stressed male offspring are tested as adults and after another period of acute stress (Estanislau & Morato, 2005).

When male offspring that were stressed prenatally were pair housed after weaning and tested as adults, they displayed less anxiety-like behavior compared to males that were not prenatally stressed (Gotz & Stefanski, 2007). The present research used social housing in the prenatal period as a potential way to decrease the anxiety behavior of offspring in the EPM. The present research also examined female as well as male offspring.

Forced Swim Test (FST). The forced swim test is an index of depressive-like behavior in rodents (Petit-Demouliere, Chenu, & Bourin, 2005). In FST, the animal swims for 15 minutes on day one, then on day two, the time the rodent spends immobile (not moving for a few seconds) is measured. This immobility is considered an index of learned helplessness because the animal has learned that it cannot escape. Learned helplessness has been applied to clinical depression because some individuals with depression perceive the absence of control over outcomes (Seligman, 1975). FST was included in the present

experiment to determine if prenatal stress alters long-term depressive-like behavior.

Many types of stressors have been used to examine the effects of stress on depressive-like behaviors in the forced swim test with mixed results. Twenty-one consecutive days of repeated corticosterone injections lead to an increase in depressive-like behaviors in the forced swim test (Gregus, Wintink, Davis, & Kalynchuk, 2005). Chronic variable stress increases depressive-like behaviors in the FST (Molina, Heyser, & Spear, 1994; Perrot-Sinal, Gregus, Boudreau, & Kalynchuk, 2004).

Prenatal stress during the last week of gestation affects immobility in the forced swim test when offspring were tested in adulthood (Van den Hove et al., 2005). Prenatal stress that lasted only one day did not affect behavior in the forced swim test in adult offspring (Frye & Wawrzycki, 2003). Yet there are other reports that rats that were prenatally stressed for longer periods of time showed increased immobility in the FST (Morley-Fletcher et al., 2003). The present experiment examined a longer period of prenatal stress (the last two weeks of the three week gestation period) and its effects on offspring behavior in FST.

Social Interaction. Social interaction is a commonly used measure in animal experiments as an index of anxiety (File & Seth, 2003). Total time spent engaged in social interaction provides an index of anxiety with decreased time spent in interaction reflecting more anxiety. There are reports that anti-anxiety drugs decrease anxiety-like behavior in a social interaction test suggesting that anxiety does indeed affect social behavior (e.g., File & Seth, 2003). Social

interaction also is a face-valid way to assess normal and abnormal rodent behaviors as well as interactions among rodents. It was included in the present experiment to assess how the offspring interacted with one another.

The effects of stress, or prenatal stress, on social interaction are not well researched. There is one report of increased anxiety-like behavior in social interaction as the result of stress in female rats during adulthood (Baranyi, Bakos, & Haller, 2005).

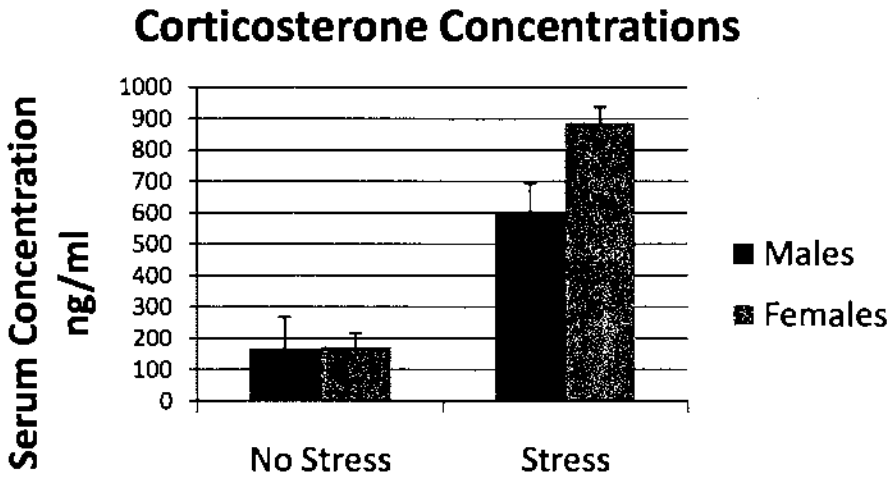
Despite the lack of research on social interaction and stress in general, there have been some investigations of prenatal stress and its effects on offspring's social interaction. The social interactions of prenatally stressed rat offspring were decreased approximately 76% relative to non-stress, control rats (Lee, Brady, Shapiro, Dorsa, & Koenig, 2007). Prenatal stress exposure also decreased the tendency to engage in social interaction behavior as adults (Lee et al., 2007; Weinstock, 2001). It appears that prenatal stress decreases subsequent social interaction.

PRELIMINARY STUDY

A preliminary study was conducted to evaluate alternate methods of manipulating stress. A common and effective method of manipulating stress is to use restraint stress (Kant, 1987; Raygada, Shaham, Nespor, Kant, & Grunberg, 1992, Acri, 1994; Faraday 2002), a finger-like restraining device that holds the animal still. In restraint stress, the "fingers," are tightened until the subjects are completely immobilized but not in apparent pain. This technique could not be used in the present experiment because the animals were pregnant and restraint stress could possibly cause physical harm to the fetus. Therefore, a preliminary experiment was conducted to determine whether the predator scent of synthetic fox urine and other unpredictable stressors (e.g., cage shaking, loud noises, bright lights) produced an increase in stress hormones. Twelve male and twelve female Sprague-Dawley adult rats (of reproductive age) were exposed to fox urine and other unpredictable stressors for 10 minutes a day for 14 consecutive days. A control group was only exposed to bright lights and 2 - 3 minutes of handling to ensure that any corticosterone effects were the result of the fox urine and unpredictable stressors and not the lights and handling.

The results are presented in Figure 1 and illustrate that the stress manipulation was effective. In fact, the effect size was greater than previous experiments in our laboratory using restraint stress (e.g., Brown et al., 2006;).

Figure 1. Corticosterone Concentrations



HYPOTHESES

The present experiment used rats to examine effects of prenatal stress on subsequent biological and behavioral variables relevant to cardiovascular disease risk. The experiment also examined if a social intervention (social enrichment) could attenuate any detrimental effects of prenatal stress. The experiment was a 2 (prenatal stress or no prenatal stress) x 2 (isolated housing or paired housing) x 2 (male or female offspring) full factorial design. The goals of the experiment were to determine: (1) the biological and behavioral consequences of prenatal stress; and (2) whether prenatal social enrichment alters the effects of prenatal stress on the offspring.

There were eight hypotheses in the present work: (1) body weight/food consumption; (2) biochemical measures of stress (i.e., corticosterone); (3) biochemical measures of cardiovascular health (serum glucose, insulin, cholesterol, and C-Reactive Protein); (4) heart structure; (5) locomotor open field activity; (6) indices of anxiety (locomotor center time and elevated plus maze); (7) index of depression (swim test); and (8) social interaction.

Hypothesis 1: Body Weight and Food Consumption

Hypothesis 1a: Stress

Prenatal stress will decrease body weight and food consumption. Body weight and food consumption decrease in response to stress (Faraday, 2002; Penke, Felszeghy, Fernette, Sage, Nyakas, Burlet, 2001; Krahn, Gosnell, Grace, & Levine, 1986).

Hypothesis 1b: Housing

Social housing environment will decrease body weight and food consumption compared with housing in an isolated environment. Environmental enrichment decreases body weight and food consumption (Brown & Grunberg, 1995; Tomchesson, 2004; Shafer, 2005).

Hypothesis 1c: Sex Differences

Males will weigh more and eat more than females. This body weight difference occurs in almost all species, including rats.

Hypothesis 2: Biochemical Measure of Stress

Hypothesis 2a: Stress

Prenatal stress will increase serum corticosterone in adult offspring. Previous research reports that stress manipulations, including fox urine and other unpredictable stressors, increase serum corticosterone (e.g., Faraday, 2000; Shafer, 2006).

Hypothesis 2b: Housing

Social housing environment will lower corticosterone levels compared with an isolated housing environment. Previous research reports that enriched environments decrease corticosterone levels in rats (Belz et al., 2003).

Hypothesis 2c: Stress and Housing

Social housing during gestation and weaning will attenuate stress-related corticosterone increases in the offspring. Previous research reports that enriched environments decrease corticosterone levels in rats (Belz et al., 2003).

Hypothesis 2d: Sex Differences

Female rats will have higher corticosterone levels than male rats as reported in previous research(e.g., Faraday, 2002).

Hypothesis 3: Biochemical Measures of Cardiovascular Health

Hypothesis 3a: Stress

Prenatal stress will increase serum glucose, insulin, cholesterol, and C-Reactive Protein levels in offspring. Previous research reports that prenatal stress (psychological or physiological) is associated with higher serum glucose (Mueller & Bale, 2006; Vallee et al., 2996) and insulin levels (Barker, 2002; Seckl & Halmes, 2007). To date, there has been no research examining the effects of prenatal stress on cholesterol and CRP levels. However, stress in adults is correlated with higher cholesterol (e.g., Sapolsky et al., 2000) and CRP levels (e.g., De Beraridis et al., 2006; Dressler et al., 2006; Taylor et al., 2006).

Hypothesis 3b: Housing

Social housing environment will decrease serum glucose, insulin, cholesterol, and C-Reactive Protein levels in adult offspring compared with offspring from an isolated housing environment. Previous research reports that social isolation leads to higher cholesterol levels in mice compared with mice in enriched environments (Bemberg, Andersson, Gan, Naylor, Johansson, & Bergstrom, 2008). There have been no studies examining the impact of environmental enrichment on glucose, insulin, or CRP levels. There also have been no studies examining the effects of prenatal enrichment on serum glucose, insulin, cholesterol, or CRP levels.

Hypothesis 3c: Stress and Housing

Social enrichment will attenuate the effects of stress to increase serum glucose, insulin, cholesterol, and C-Reactive Protein levels. Social support decreases levels of CRP in humans exposed to early-life stressors (Taylor et al., 2000).

Hypothesis 4: Heart Morphology

Hypothesis 4a: Stress

Prenatal stress will change the structure of the offspring's heart, such that offspring with prenatal stress will have reduced heart lengths and left ventricle cavity widths and thicker septal walls. Previous research reports that restraint stress in rats (during adolescence or adulthood) causes decreases in heart lengths and increases in septal wall thickness (Elliott et al., 2004; Shafer, 2006). There are human reports that psychological stress is positively correlated with left ventricular mass (e.g, Sherwood, Gullette, Hinderliter, Georgiades, Babyak, & Waugh, et al., 2002).

Hypothesis 4b: Housing

Social housing environment will decrease septal wall thickness and increase heart length as is consistent with previous reports (Shafer, 2006).

Hypothesis 4c: Stress and Housing

Social housing will attenuate the effects of prenatal stress on the heart. Enrichment in adolescent rats attenuates the stress response on the heart (Shafer, 2006).

Hypothesis 4d: Sex Differences

Male offspring will have longer hearts and thicker left ventricular walls compared with female offspring, consistent with previous research (Elliott et al., 2003).

Hypothesis 5: Open Field Locomotor Behavior

Hypothesis 5a: Stress

Prenatal stress will decrease activity levels in the locomotor open field chamber compared with rats not exposed to prenatal stress. Previous research reports that stress decreases horizontal activity open field chamber (Faraday, 2002).

Hypothesis 5b: Housing

Social housing will decrease horizontal activity compared with an isolated housing environment, consistent with previous research (Tomchesson, 2005; Grunberg et al., 2004)

Hypothesis 6: Anxiety-Like Behavior

Hypothesis 6a: Stress

Prenatal stress will increase anxiety-like behaviors (as assessed by decreased center time in an open field chamber and decreased time spent in the open arms) compared with offspring not exposed to prenatal stress. Previous research reports that stress decreases time spent in the open arms of Elevated Plus Maze (EPM) and center time in open field (Adamec et al., 2006; Benaroya-Milshtein et al., 2004; Imanaka et al., 2006).

Hypothesis 6b: Housing

Social enrichment will decrease anxiety-like behaviors. Previous research reports that enrichment increases time spent in the open arms of EPM (e.g., Schmitt & Heimke, 1998).

Hypothesis 6c: Stress and Housing

Social enrichment will attenuate the effects of prenatal stress on anxiety. Human research has shown that social support expedites treatment for anxiety disorders (Flaherty et al., 1983; Houston et al., 2002).

Hypothesis 6d: Sex Differences

Female rats will have more anxiety-like behaviors than male rats. Women have higher rates of anxiety and depression than men (WHO, 2008).

Hypothesis 7: Depression-Like Behavior

Hypothesis 7a: Stress

Prenatal stress will increase depressive-like behaviors (as assessed by increased immobility in the forced swim test) compared with rats not exposed to prenatal stress. Stress, depression, and prenatal stress increase immobility FST (Cui et al., 2006; Hattori et al., 2007; Abe, Hidaka, Kawagoe, Odagiri, Watanabe, & Ikeda et al., 2007).

Hypothesis 7b: Housing

Social housing environment will decrease depressive-like behaviors compared with isolated housing. Social enrichment decreases immobile time in FST (Brenes, Rodriguez, & Fornaguera, 2008)

Hypothesis 7c: Stress and Housing

Social enrichment will attenuate the effects of prenatal or early life stress on depressive behaviors. Human research has shown that social support expedites treatment for depression (Flaherty et al., 1983; Houston et al., 2002).

Hypothesis 7d: Sex Differences

Females will have greater depressive behaviors than males. Women have higher rates of anxiety and depression than men (WHO, 2008).

Hypothesis 8: Social interaction

Hypothesis 8a: Stress

Prenatal stress will decrease overall social interaction and increase negative social interactions. Prenatal stress decreases social interaction behavior (Lee, Brady, Shapiro, Dorsa, & Koenig, 2007; Weinstock, 2001). Reports from human research also indicate that there is a correlation between prenatal stress and externalizing behaviors (e.g., aggression/destruction) at age 2 (Robinson, Oddy, Li, Kendall, de Klerk, & Silbum et al., 2008).

Hypothesis 8b: Housing

Social housing will increase positive social interaction behaviors. Enrichment in mice leads to more affiliate social interactions than isolation (Pietropaolo, Branchi, Cirulli, Chiarotti, Aloe, & Alleva, 2004).

Hypothesis 8c: Stress and Housing

Social environment will attenuate the decrease in social interaction that occurs because of stress. Enrichment in mice leads to more affiliative social

interactions than isolation (Pietropaolo, Branchi, Cirulli, Chiarotti, Aloe, & Alleva, 2004).

Hypothesis 8d: Sex Differences

Females will have less total social interaction behaviors than males, based on previous research using this paradigm of social interaction (Johnston & File, 1991).

METHODS

The purpose of the present research was to examine the long-term biological and behavioral effects of prenatal stress and social enrichment on variables relevant to heart disease. The subjects were 80 offspring from 14 dams (i.e., pregnant rats). The experiment was a 2 x 2 x 2 full factorial design with prenatal stress, prenatal and weaning social enrichment, and offspring sex (male or female) as the independent variables. Dams were randomly assigned to a stress or no stress condition and to an isolated or paired (social enrichment) housing condition upon arrival. After the dams delivered, the pups remained with the dam until weaning at postnatal day 22 (PN22) (see Table 2). On PN Day 22 the pups were weaned and then individually housed for the duration of the experiment. Over the next four months, the offspring matured into adulthood and were assessed for biological and behavioral dependent variables relevant to cardiovascular disease. The biological variables included: body weight, serum corticosterone, cholesterol, C-Reactive Protein, serum glucose, insulin, and heart morphology. The behavioral variables included: food consumption, open field locomotor activity (horizontal activity and center time), elevated plus maze, forced swim test, and social interaction. Offspring also were divided into two cohorts (an even split between experimental conditions) that were staggered by one day so that behavioral assessment was logistically possible.

Subjects and Housing

The initial subjects were 14 pregnant Sprague-Dawley dams that were approximately 65 days old and four days into their three-week gestation period at

the beginning of the experiment (Charles River Laboratories). Sprague-Dawley albino rats were used because they are the most commonly used laboratory rats in stress studies and other experiments (Suckow, Weisbroth, & Franklin, 2006). This number (14) of dams was used because it was estimated that each dam would have a litter of 6 - 12 pups and that half of each litter would be male and half would be female. Therefore, the 14 dams were estimated to give birth to 84 - 168 pups (42 - 84 of each sex), most (but not all) of which were expected to survive. The experimental design included eight conditions with 10 subjects per condition for a total of 80 pups (40 of each sex). When they arrived, half the pregnant dams (n = 6) were housed alone in cages (42.5 x 20.5 x 20 cm) with Pine-Dri bedding and unlimited access to water and food (Harlan Teklad 4% Mouse/Rat Diet 7001). These housing and food/water conditions are common and previously reported in several studies including Shafer (2006) and Perry (2009). The remaining dams (n = 8) were pair-housed in large cages (46 cm x 36 cm x 20 cm) with similar bedding, food, and water. Half of the dams in each housing condition were stressed. The pair housed condition had two extra dams for two reasons: (1) the rats housed together needed to be in the same stress condition (i.e., both in the stress or no-stress condition); and (2) a total of six dams would yield three pairs leaving an uneven number of pairs in the stress and non-stress conditions. Eight dams yielded four pairs, allowing two pairs for the non-stress condition and two pairs for the stress condition.

All dams delivered within the same 24 hour period, 18 days after their arrival (rat gestation lasts for approximately three weeks). Rat weaning is

estimated to be about 21 days. Therefore, the pups remained housed with their dams for 22 days after birth. Then, the pups were "sexed" (i.e., their sex was determined by observation of anogenital distance with females having a smaller anogenital distance) and 80 pups were individually housed in standard cages for the remainder of the experiment. It was intended that there would be 40 males and 40 females in the experiment. However, rat sex is determined by ano-genital distance and cannot be completely confirmed until puberty when the testes descend in the male rat. As a result of the late confirmation of offspring sex, there ended up being 42 males and 38 females in the experiment.

After weaning, the rats matured into adolescence and adulthood. Rat adolescence has been defined as 21-42 days old and up to day 55 for male rats (Spear & Brake, 1983). During adolescence, rats share characteristics that are similar to human adolescents in that they spend a greater amount of time "playing" with their peers and taking risks than adult rats (Varlinskaya, Spear, & Spear, 1999). Rat adulthood begins around day 55 and life expectancy is around 730 days (about 2 years). The rats in this study were followed until postnatal day 150 (about 5 months) which is considered young adulthood. All of the behavioral assessments occurred during adolescence and adulthood to account for differences in behavior throughout development.

The dams gave birth to approximately 124 pups. The exact number of pups at birth could not be determined because dams naturally keep their litters in a pile making it hard to count. In this experiment, the dams and pups were left completely undisturbed until postnatal day 17 to minimize any additional stress.

There were at least two known deaths of newborn pups. There may have been other deaths, but because dams often eat deceased or sick offspring, exact numbers could not be determined. It is interesting to note that both confirmed deaths and a rat born with only one eye all occurred in the stress/social condition. Below is a table of the approximate number of pups born in each condition and their average weight on PN Day 17 (before weaning).

Table A. Information about Birth of Pups

PUP BIRTH INFORMATION					
Condition	**Number of Dams**	**Approx. Total # of Pups for Condition**	**Approx. # of Pup Deaths**	**Mean Weight on PN Day 17**	**Std. Error of the Mean**
No Stress/ Isolated	3	21	0	21.2	0.45
Stress/Isolated	3	31	0	16.2	0.68
No Stress/Social	4	32	0	19.4	0.47
Stress/Social	4	36	2	13.9	0.58

Note: There is a significant prenatal stress main effect and a significant prenatal housing effect for mean weights on postnatal day 17.

The pups that did not participate in the experiment either were used in other experiments or were euthanized by the Uniformed Services University Laboratory of Animal Medicine personnel by CO_2 inhalation. The housing room (for the subjects in the experiment) was maintained at room temperature of 23^0 C with a humidity of approximately 50% and a 12-hour (0600-1800 hours) reverse light cycle.

Stress

Stress manipulation in animal experiments varies greatly (e.g., electric shock, crowding, cold water immersion, predator, intruder, restraint). The present experiment used a variable stressor that has been previous studies (e.g.,

Berger and Grunberg [in preparation], Perry 2009). The procedure included exposure of pregnant dams to a foxurine, unpredictable lights, noises and cage-shaking in a cage that resembles (but is not) the housing case. This procedure occurred in a room that was similar to, but separate from, the housing room. For rats, fox urine is a scent from a predator that leads to corticosterone release (e.g., Hayley et al., 2001; Berger & Grunberg, in prep).

During the procedure, the animals were transferred from their home cage to a "stress cage" (42.5 x 20.5 x 20 cm). This cage was covered with a lid, but did not contain bedding. The stressor was 10 minutes in duration and began (2 ½ hours after the "off" phase of the light cycle (i.e., morning for the animals). Fox urine (15 mL) was absorbed by a cotton ball and positioned in varying locations of the cage. During the fox urine exposure, the room was lit with a standard, overhead florescent light. placed on a large cotton ball and placed in varying spots in the stress cage. See Table B for a description of the unpredictable stressors that were given in addition to the fox urine.

Table B. Unpredictable Stressors Occurring with Scent of Fox Urine

Stress Day	Stressor	Description
1	Fox Urine Only	
2	Urine + Sound 1	Old Fashioned alarm clock at 3, 5, and 8 minutes
3	Urine + Light 1	100 W directly over rat cage for duration of stress
4	Urine + Sound 2	Short blow of traditional whistle at 2, 6, and 8 minutes
5	Urine + Cage Shaking	Vigorous cage shaking at 4 and 6 minutes
6	Urine + Sound 3	6 short blows of a whistle at 2 and 6 minutes
7	Fox Urine Only	
8	Urine +Cage Shaking	(see above)
9	Urine + Light 2	Overhead, fluorescent lights flash at 2, 6, and 9 minutes
10	Fox Urine Only	
11	Fox Urine + Sound 4	Shaking of coins in a metal container at 3 and 7 minutes
12	Urine + Sound 3	(see above)
13	Urine + Cage Shaking	(see above)
14	Fox Urine Only	

During the stress procedure, the non-stress group was placed in a room that was separate from their housing room (but remained in their home-cage) while the stress group underwent the stress procedure. When the stress procedure ended, the experimenters changed lab coats and gloves and gentled the non-stress group (to avoid any possible scent of fox urine or stress hormones). Both groups were returned to housing room after their procedures. This stress procedure was a based on a preliminary experiment conducted by

Berger and Grunberg (in prep) that found the procedure to be effective in increasing corticosterone concentrations in sexually mature male and female rats (see "Preliminary Study" section).

Biological Dependent Variables

Body Weight

Body weight is relevant to many physical and mental health conditions (e.g., anxiety, depression, eating disorders, obesity, cardiovascular diseases) and is used in many rodent experiments as a measure of general health or to determine the effect of various manipulations on the animal (Suckow et al., 2006).

For the offspring, body weight was measured once per week throughout the experiment with the exception of more frequent measurements after weaning to ensure that weight gain occurred. Animals were removed from their cages and gently placed in a weighing pan on an electronic scale (Sartorious electronic scale). To ensure accurate weight measurements (i.e., reduce measurement error) the electronic scale automatically obtained multiple weight readings and provided an average of these readings. Early weights of the pups also were assessed when they were still with the dam at PN Day 17.

Blood and Tissue Sample Collection

On postnatal day 150, the rats were individually anesthetized using CO_2, then immediately decapitated. All decapitations occurred in the morning and the order was counterbalanced by experimental condition. In other words, two animals from the non-stress/isolated/male condition were decapitated, followed

by two animals from the non-stress/isolated/female condition, followed by two animals from the stress/isolated/male condition and so forth. Trunk blood was taken from the animals and immediately place in a non-heparinized tube and put on wet ice. Within 30 minutes of decapitation, the blood was spun in a refrigerated centrifuge (4° C) at 2500 rpm for 20 minutes. Serum was removed from the non-heparinized tubes using disposable pipettes and placed into smaller tubes. These smaller tubes were frozen at -80° C for later assay.

Immediately after decapitation, the rats' hearts were removed from the chest cavity using a scalpel. The hearts were immediately placed into a vial containing 10% buffered formalin phosphate for later analyses.

Serum Corticosterone

The hypothalamic-pituitary-adrenal (HPA) axis is activated in response to a stressor. HPA activity can be assessed by examinations of serum concentrations of several biochemicals, including corticosterone (e.g., Faraday, 2002; Selye, 1973). This measure was included in the present experiment to determine if prenatal stress and prenatal enrichment have long-term effects on the stress biochemistry in the offspring.

In this experiment, corticosterone was assayed using an ImmuChem Double-Antibody radioimmunoassay (RIA) kit with ^{125} I-labeled corticosterone (MP Biomedicals). This procedure was performed in the laboratory of Neil E. Grunberg at the Uniformed Services University of the Health Sciences (USUHS). As described in Faraday (2002), Shafer (2006), and Perry (2009), this procedure involves using a limited amount of specific antibody that reacts with a fixed

quantity of ^{125} I-labeled corticosterone. The concentration of unlabeled corticosterone in samples increases as a function of the decreasing percentages of bound radioisotope-labeled corticosterone. A second antibody precipitates antibody bound to antigen. All samples and standards were run in duplicate. The coefficient of variation of the assay is 6.93% and the sensitivity is 8 ng/ml and the (Faraday, 2002).

Serum glucose

Serum glucose is the assessment of the glucose (sugar from carbohydrates) in the blood and can be obtained after a period of fasting or after an individual has consumed food (Mayo Clinic, 2007). There is evidence that higher serum glucose levels over time increase risk for heart disease even if an individual does not have diabetes (Kanaya, Grady, & Barrett-Connor 2002).

Serum glucose was assayed by Diagnostic Services and Comparative Medicine at USUHS using a VITROS GLU Slide methods using VITROS GLU Slides and the VITROS Chemistry Products Calibrator Kit 1 on VITROS Chemistry Systems. The VITRUOS GLU slide is a multilayered, analytical element coated on a polyester support. A drop of sample was deposited on the slide and evenly distributed by the spreading later to the underlying layers. The oxidation of the sample glucose was catalyzed by glucose oxidase to form hydrogen peroxide and gluconate. After this reaction, there is an oxidative coupling and production of a dye. This dye was measured by reflected light. The coefficient of variation for this assay is 1.7% and the sensitivity is 20mg/dl.

Insulin

Insulin is a hormone produced by the pancreas that allows glucose to enter the cells. Insulin resistance is a generalized metabolic disorder, in which the body cannot use insulin efficiently. Insulin resistance, combined with the other risk factors of obesity and elevated lipids are known to increase the risk of cardiovascular disease (American Heart Association, 2007).

Serum insulin was assayed using RIA kits purchased from MP Biomedicals in the laboratory of Neil E. Grunberg at USUHS. In this procedure, the antibody was covalently bound to the inner surface of a polypropylene tube. Therefore, an antibody-bound antigen complex was also bound to the tube wall. At the end of the assay procedure, free antigen was aspirated, leaving only antibody-bound antigen. The coated tube was then counted in a gamma counter. The coefficient of variation in this assay is 5.0% and the sensitivity of is 4.6 μl/mL.

Cholesterol

Cholesterol is a lipid in the body's cells and is carried in the blood. Abnormally high cholesterol levels and abnormal proportions of low-density lipoprotein (LDL) and high-density lipoprotein (HDL) increase cardiovascular disease risk because they can increase atherosclerosis (American Heart Association, 2007). As a result of cholesterol's role in cardiovascular risk, it was included in the present experiment.

Cholesterol was assayed by Diagnostic Services and Comparative Medicine USUHS using a VITROS CHOL Slide methods using VITROS CHOL

Slides and the VITROS Chemistry Products Calibrator Kit 2 on VITROS Chemistry Systems. The VITRUOS CHOL slide is a multilayered, analytical element coated on a polyester support. The method is based on a previously used enzymatic method. A drop of sample was deposited on the slide and evenly distributed by the spreading later to the underlying layers. After several oxidization processes, hydrogen peroxide produces a colored dye. The dye formed is measured by reflectance spectophotometry. The coefficient of variation for the assay is 1.3% and the sensitivity is 50 mg/dl.

C-Reactive Protein

The C-Reactive Protein (CRP) assay was conducted in the Behavioral Neuroimmunomodulation Laboratory at Pennsylvania State University using an enzyme-linked immunosorbant assay (ELISA) kits purchased form Immunology Consultants Laboratory, Inc. In this assay, the CRP in the samples mixes with anti-CRP antibodies in microtitre wells. Then, washing removes the unbound serum proteins and anti-CRP antibodies are added. The amount of CRP in the test sample can be determined by using the assay's standard curve. The coefficient of variation of this assay is 1.7% and the sensitivity is 6.25 ng/mL.

Heart Morphology

Heart morphology is the assessment of the heart's dimensions (e.g., left ventricular size, length, width, weight, etc). Stress and enrichment have been reported to affect heart morphology in rats (Elliott et al., 2003; Shafer, 2006)

The heart morphology analysis procedure was the same procedure used and described in Elliott and colleagues (2003) and Shafer (2006). Digital calipers were used to measure the length of each heart (from base to apex). Cross-sectional slices of the heart were made through the ventricles (midway between the apex and base of the heart) using a scalpel. Measurements were made of the left ventricle, right ventricle,, anterior wall, posterior wall, lateral wall, and septal wall. Two observers measured each heart. If the inter-rater reliability was less than 0.90, then a third person measured the heart and the two closest measurements were used in the analyses.

Behavioral Dependent Variables

Food Consumption

Food consumption was used as a measure of general health and to determine the effect of stress and social enrichment on the animal. Stress (Faraday, 2002; Levine & Morley, 1981; Grunberg & Straub, 1991) and environmental changes (Tomchesson, 2005; Shafer 2006) affect food consumption.

Food consumption was measured every week for the offspring after they were weaned and started on solid rat chow. Food pellets were placed on the top of each cage and animals had continuous access to food. Food consumption was determined by weighing the animal's food tray using an electronic scale (Sartorious electronic scale), then subtracting that day's value from the previous value (e.g., subtracting Day 16 food weights from Day 14). When new food was

added, the new weight was recorded and this new weight was used in the next calculation.

Open Field Activity (OF)

Open field locomotion refers to an animal's behavior when placed in a non-home cage arena (see Figure 2). Animal behaviors in the open field have been used as measures of general locomotion, exploration, and anxiety or stress responses. For the present experiment, the activity domains of interest were horizontal activity and center time. Horizontal activity provides an assessment of general activity level. Center time is an index of anxiety. A greater amount of center time is an index of less anxiety-like behavior. Open field activity was measured on postnatal days 24 and 25; 48 and 49; 83 and 84; 128 and 129. The open field activity procedure was the same procedure used and described in Elliott and colleagues (2003), Shafer (2006), Perry (2009) as well as other researchers. Open-field activity was measured using an Omnitech/Accuscan Electronics Digiscan infrared photocell system (Test box model RXYZCM [16 TAO]); Omnitech Electronics, Columbus, OH) in a room that was separate from the housing room. Animals were placed singly in a 40 x 40 x 30 cm clear Plexiglas arena with a Plexiglas lid that had multiple 3.5 cm diameter holes on top of the arena (see Figure 2 in the Appendix [Figures 2-5 are photographs that appear in the Appendix]). A photocell array measured horizontal activity using 16 pairs of infrared photocells located every 2.5 cm from side-to-side and 16 pairs of infrared photocells located front-to-back in a plane 2 cm above the arena floor. Data were transmitted to a computer via an Omnitech Model DCM-I-BBU

analyzer. Once subjects were placed in the test arenas, the experimenter turned off the lights and left the room. Animal activity was assessed for 60 continuous minutes.

Elevated Plus Maze (EPM)

Elevated Plus Maze is commonly used to index anxiety in rodent research (Elliott, Faraday, Phillips, & Grunberg, 2004; Pellow, Chopin, File, & Briley, 1985; Hogg, 1996; Kalinichev et al., 2002). The apparatus consists of four radiating platforms that are at right angles to each other (see Figure 3). Two of the arms have high walls that enclose the platforms; two of the arms have no walls. Each subject was initially placed in the center of the maze facing toward the front open arm of the maze and allowed to explore the maze for 5 minutes. Time and entries into the open and closed platform arms were recorded using AnyMaze software®. This task does not require training, food or water deprivation, or aversive stimuli. It was easy to conduct and took 5 minutes per animal. A variety of species have been used in the elevated plus maze, including rats (Pellow, Chopin, File, & Briley, 1985), mice (Listar, 1987), and guinea pigs (Rex, Fink, & Marsden, 1994). The EPM is bidirectionally sensitive to anxiety manipulations and anxiety-like responses. Therefore, EPM is sensitive enough to detect both increases and decreases in anxiety. The two primary indices of anxiety in the EPM are the percentage of time spent in the open arms and the percentage of entries into open arms, where more time in open arms is interpreted as less anxiety.

Elevated plus maze was measured on either postnatal days 28 or 29 and 134 or 135, depending on the experimental cohort. The EPM apparatus was built following the basic plus maze design of Pellow (1985). It has four arms radiating out from a central square platform and it looks like a large plus sign from above (also referred to as t shaped). It is elevated 60 cm above the floor. Two of the four arms have black opaque sidewalls (50 cm in height), while the remaining two arms have a short, block, wood ledges (about 2 inches high) that are only there to keep the animal from falling off the maze without providing tall walls or enclosures (see Figure 3). These two types of arms (enclosed and non-enclosed) are at right angles to each other and are generally referred to as closed and open arms, respectively. EPM was conducted in a dedicated room (with cinder block walls) where outside sound was kept to a minimum and environmental lighting was provided by a six-foot floor lamp with a 40-watt light bulb placed approximately 15-feet from the EPM and pointed away from the apparatus. Other than this lamp, the lights in the testing room remained in the off position, therefore, the EPM room was illuminated at 4.30 lx (Advanced Light Meter, Model No. 840022, Sper Scientific Ltd.). Elevated plus maze activity was recorded using a video camera and a commercially available software tracking system (AnyMaze®).

Forced Swim Test (FST)

The forced swim test is a common rodent behavioral test for depression (Petit-Demouliere et al., 2005) (see Figure 4). The FST was measured on postnatal days 76-79 and 136-139. The procedure was the same as the

procedure using in Perry (2009) and involved a two-day process. Rats were put into a cylindrical container (65 cm x 25 cm x 48 cm) that contained room temperature water. On Day 1, the rats swam for 15 minutes (unless visibly struggling to keep their head about water, which did not occur). On Day 2, the rats were re-assessed for 5 minutes under the same conditions to determine the latency to become immobile (i.e., the rat momentarily stops using its front paws to remain afloat). This immobility is an index of learned helplessness, which is thought to be a symptom of clinical depression (Seligman, 1975). Immobility was assessed by AnyMaze® software. On both days, after the designated time, the rats were removed from the water and warmed with towels and a warming lamp.

Social Interaction

Social interaction was measured on postnatal days 34 or 35 and 124 or 125. Two animals (one from each condition) were placed in opposite corners of a novel arena (40 x 40 x 30 cm clear Plexiglas arena with a Plexiglas lid with multiple 3.5 cm diameter holes on top of the arena) for 10 minutes (see Figure 5). Animals were watched by condition-blind, trained graduate students and research assistants and behaviors at 15-second intervals were recorded by observers. Animal social interactions also were videotaped in case there were discrepancies in ratings; however, no discrepancies were found.

Three categories of behaviors were observed in social interaction: exploratory behaviors, social behaviors, and behaviors that were not classified in either of those groups were termed "other." The exploratory behaviors included freezing, sniffing, moving, moving and sniffing (occurring simultaneously), and

rearing. The social behaviors included touching, following, sniffing other rat, wrestling, boxing/biting, or grooming other rat. The "other" behaviors included grooming self and eating. Three values were used in the analyses of social interaction: total social behaviors; total instances of positive behavior (grooming was the only positive behavior quantified); and total instances of negative behavior (boxing/biting was the only negative behavior quantified) (File & Seth, 2003; Scheufele, Faraday, & Grunberg, 2000).

Sample Size Determination

The sample size for each cell was 10 animals. This size was calculated using previous reports in the areas of stress and environmental enrichment literature (e.g., Tomchesson 2005; Elliott et al., 2003) and by conducting a power analysis.

Enrichment experiments report statistically significant effects with 7 – 12 animals per cell (e.g., Van Praag et al., 1999; Passineau et al., 2001; Elliott & Grunberg, 2003; Tomchesson, 2005) and 8-20 animals per cell have been used to achieve effects of prenatal stress (Glavin, 1984; Koehl et al., 1999). Mering, Kaliste-Korhonen, and Nevalainen (2000) determined that 5 - 10 animals were needed to find statistically significant effects for enrichment on various biological measures (e.g., body weight, blood chemistry).

As previously described in Tomchesson (2005), Shafer (2006), Perry (2009) and other work in the Grunberg laboratory, measures of body weight, food consumption, corticosterone, insulin, heart morphology, locomotor activity, and elevated plus maze are well established and have shown significant effects and

power of at least 0.80 in sample sizes of 8 subjects or more. The power and sample size for cholesterol, serum glucose, C-Reactive Protein, forced swim test and social interaction were determined with computer programs (Java applets for power and sample size). Zardooz, Zahedi Asl, & Nasweri (2006) reported using 8 animals/cell and a large effect size of 1.9 in a study that examined stress and glucose. Estimating 10 animals/cell in the present experiment with an effect size of 1.5, the power for glucose was calculated to be at 0.99. Shalyapina et al. (2007) reported using 12 animals/cell and a large effect size of 1.5 in a study that examined forced swim test effects. Estimating 10 animals/cell in the present experiment with an effect size of 1.5, the power was calculated to be at 0.94. The effects of psychological stress on CRP in rats have not been examined to date; therefore, estimates for power could not be determined. The results of stress effects on swim immobility reported by yielded a large effect size of 1.5 with a sample size of 12 animals per cell. Using 10 animals per cell in the current experiment and an effect size of 1.5, the power for swim test immobility was predicted to be 0.94.

Procedure

The experiment began with a gestation phase in which the dams were one week into the gestation period (gestation in a rat is approximately three weeks). During the gestation phase, the dams were singly housed in cages measuring 40 cm x 20 cm x 20 cm (Isolated Condition) or pair housed in larger cages measuring 46 cm x 36 cm x 20 cm (Social Enrichment Condition). The dams remained in the assigned condition through delivery and weaning of the pups

(until postnatal day 22). Assessment of offspring began three weeks after birth, which also was after weaning. The offspring were housed with the dams until fully weaned at 22 days. On postnatal day 22, the rats were removed from the cages, weighed, and housed individually where they remained until the end of the experiment.

The experiment ended on postnatal day 150 (approximately 5 months). During PN Days 22-150, the offspring matured into adulthood and the behavioral dependent variables (e.g., open field, social interaction) and body weight were assessed (see Table 2 for timeline). Animals were tested individually for all behavioral measures except social interaction where there were two animals in the arena at a time (animals were paired within experimental condition – e.g., a non-stress, isolated female was paired with another non-stress, isolated female). Behavioral measures were conducted during the dark cycle (the active cycle for the rats). This period of time was used so that behavioral performance and activity were maximized.

The experiment was conducted in two cohorts (A and B) of 40 animals each for logistical reasons. Animals were counterbalanced by condition and then randomly assigned to Cohort A or Cohort B with the cohorts having identical numbers of animals from each condition. Cohorts A and B had an identical schedule until postnatal day 24 when cohort B underwent behavioral testing 1-2 days later than cohort A for every measure. For example, open field was assessed on postnatal day 24 for cohort A, and postnatal day 25 for cohort B (see Table 2 and other tables in the Appendix).

Data Analytic Strategy

Pregnant dams were randomly assigned to experimental conditions. Different data analytic strategies were employed depending on the dependent variable.

Separate analyses of variance (ANOVAs) were used to analyze serum corticosterone, serum glucose, insulin, and C-Reactive Protein. Heart morphology was analyzed by MANCOVA with body weight as a covariate. Multivariate analyses were used because the various heart measurements (e.g., left and right ventricle) were statistically and conceptually correlated.

Repeated-measures ANOVAs were used to analyze body weight (BW) and food consumption (FC) because it allows for an examination of how BW/FC changed throughout the experiment. At times, separate ANOVAs were used to analyze BW during critical times in the experiment (e.g., weaning weight). When there were significant main effects or interactions, separate ANOVAs were used for further analysis (as described by Keppel,1991).

For open field activity, there were two indices of interest – general horizontal activity and center time. General horizontal activity was analyzed using a repeated-measures ANOVA to determine whether activity differed among groups and over time. Center time was analyzed using separate ANOVAs for each time point (four open field assessments) to determine if there were differences among the groups in anxiety at different points in their development. Center time also was analyzed using a center time ratio, which was calculated by dividing center time by overall movement time. These ratios also were analyzed

using separate ANOVAs for each time point. Elevated Plus Maze, Forced Swim Test, and Social Interaction were analyzed using separate ANOVAs for measurements during adolescence and adulthood.

Eta-squared values were used to determine the relative magnitude of enrichment effects for each group. Eta-squared is a measure of effect size that indicates the proportion of variance in a dependent variable explained by a given independent variable (Cohen, Cohen, West, & Aiken, 2003). All tests were two-tailed with significance determined by $p \leq 0.05$ unless otherwise noted.

Five main approaches were used to decrease the probability of statistical error (i.e., Type I and Type II error): (1) experimental design; (2) a priori hypotheses; (3) conservative use of internal analysis; (4) use of MANOVAs or repeated-measures ANOVAs when possible; (5) consideration of the goals of the experiment. Each of these approaches is described in detail below. First, the experiment was designed with sufficient power (0.80) which reduces the probability of Type II error. Second, all analyses were based on a priori hypotheses which reduces the probability of Type I error. Third, for the most part, internal analyses were only conducted when the overall analysis revealed a significant effect thereby decreasing the number of analyses performed and the probability of Type I error. Fourth, when possible, MANOVAs or repeated-measures ANOVAs were conducted, again, reducing the number of tests performed and therefore decreasing the probability of Type I error. Fifth, the goals of the experiment also took into consideration that the number of tests run could lead to a slight increase in the probability of Type I error. The goals of the

experiment were to explore different ways in which prenatal stress and prenatal enrichment affect long-term cardiovascular disease. The goal was not to change clinical guidelines that could profoundly affect patient health. In this study, the consequence of Type I error could be that a statistically significant result was detected when one did not truly exist. This potential effect could provide specific directions for future research and areas to replicate. If traditional, statistical corrections (e.g., Bonferroni) were used to correct for the multiple tests, then some of the effects might not be detected. As a result, future studies might only focus on the powerful effects and neglect some of the subtler effects that might need more research attention.

RESULTS

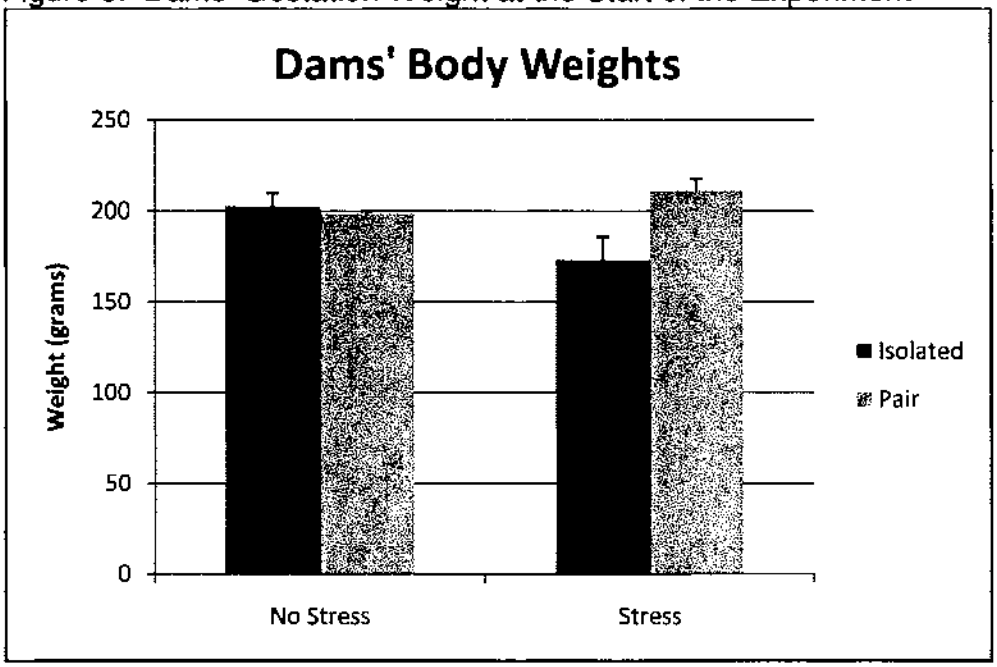
The following abbreviations are used in tables and equations throughout the Results and Discussion sections. For the three independent variables: offspring's dam not prenatally stressed (NS); offspring's dam prenatally stressed (St), offspring's dam housed in isolation during gestation and weaning (I), offspring's dam housed with social enrichment (i.e., with another dam) during gestation and weaning (S), offspring was male (M), offspring was female (F). The following abbreviations are used to reference the eight experimental groups: non-stress/isolated/male (NSIM), non-stress/isolated/female (NSIF), stress/isolated/male (StIM), stress/isolated/female (StIF), non-stress/social/male (NSSM), non-stress/social/female (NSSF), stress/social/male (StSM), stress/social/female (StSF). Figures are displayed within the text of each section. Six tables (labeled Tables A - F) are displayed within the text and are labeled alphabetically. Tables of descriptive statistics and analyses are in the Appendix and are labeled numerically. The order of values among groups is represented by mathematical relationships (e.g., StIF > NSIF> NSIM > NSIM). Single asterisks are used to indicate a statistically significant difference between two or more particular groups (e.g., StIF* > NSIF*> NSIM > NSIM). Double asterisks similarly are used to indicate a statistically significant difference between two groups (e.g., StIF* > NSIF*> NSIM** > NSIM**).

Biological Variables

Body Weight

The dams were weighed throughout gestation with their first assessment occurring less than 24 hours after arrival. There was a significant difference between the housing conditions ($F [1, 10] = 5.39$, $p < 0.05$) with the social dams weighing more than the isolated dams. There also was a significant stress x housing interaction ($F [1, 10] = 8.05$, $p < 0.05$) with the dams in the prenatally stressed and social condition weighing more than the dams in all other groups (StS* > NSI > NSS > StI*) (see Figure 6 and Tables 4 and 5).

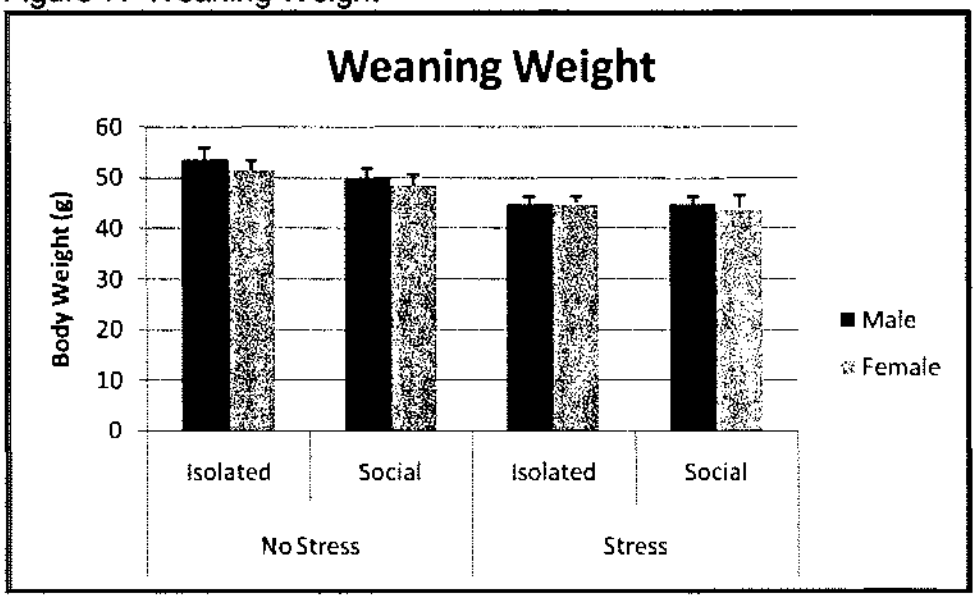
Figure 6. Dams' Gestation Weight at the Start of the Experiment



On weaning day (postnatal day 22 – the offspring were removed from the dam's cage and housed individually), there was a significant main effect for prenatal stress ($F [1, 71] = 22.39$, $p < 0.01$) such that non-stress rats (i.e., offspring) weighed more than stressed rats and this was true for both sexes (i.e.,

offspring of the stressed dams) (see Figure 7 and Tables 6 and 7). There were no significant main effects of housing or sex or any significant interactions.

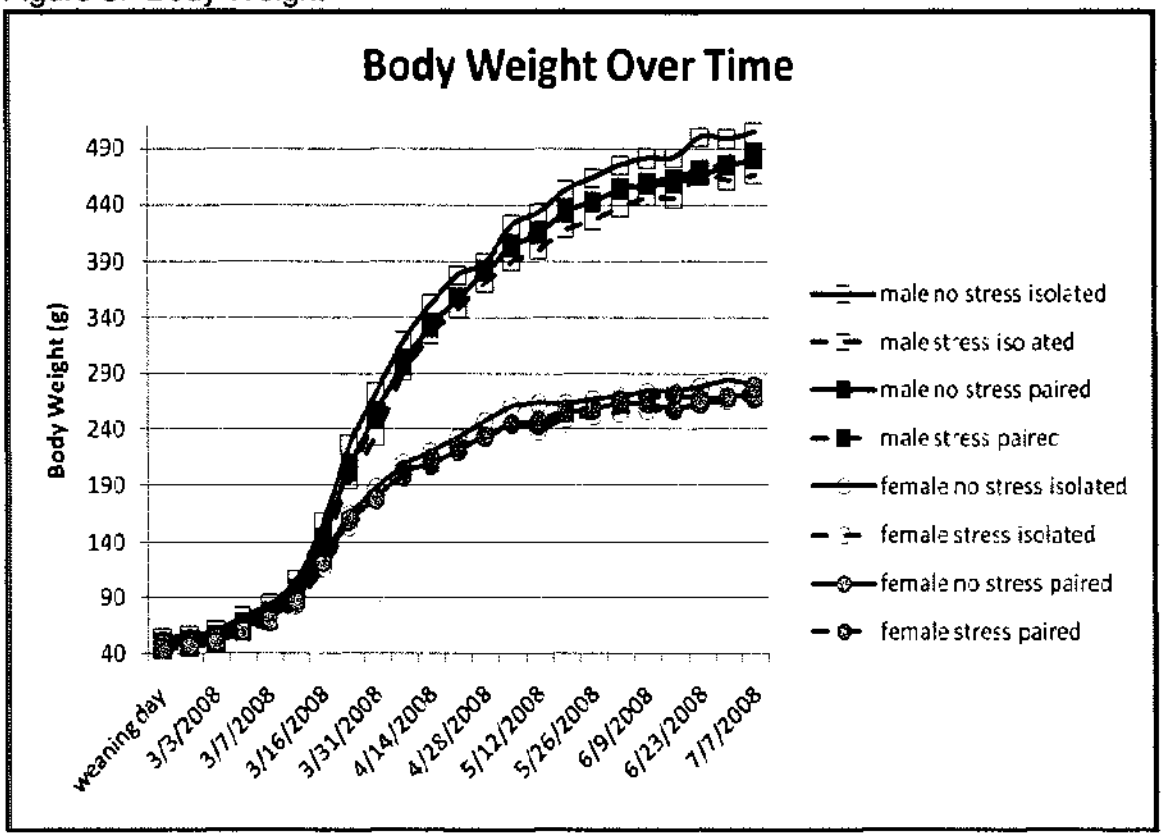
Figure 7. Weaning Weight



A repeated-measures ANOVA was conducted beginning on postnatal day 23 and ending on postnatal day 150 (the last day of the experiment). Animals that were prenatally stressed weighed less than non-stress animals ($F [1, 71] = 4.84$, $p < 0.05$) and males weighed more than females ($F [1, 71] = 383.8$, $p < 0.01$). An internal analysis of sex revealed that males were responsible for the stress effect ($F [1, 37] = 3.15$, $p = 0.08$) as there was no main effect of stress for females (see Tables 8a-b and 9). There was a stress x housing interaction ($F [1, 71] = 3.11$, $p < 0.05$) with non-stress/isolated rats weighing the most and stress/isolated rats weighing the least (NSI > NSS* = StS* > StI*). There also was a main effect of time with body weight increasing over time ($F [3, 213] = 2303.3$, $p < 0.01$) for both sexes (see Figure 8). There also were interactions with time: time x stress ($F [3, 213] = 2.77$, $p < 0.05$) with non-stress rats gaining

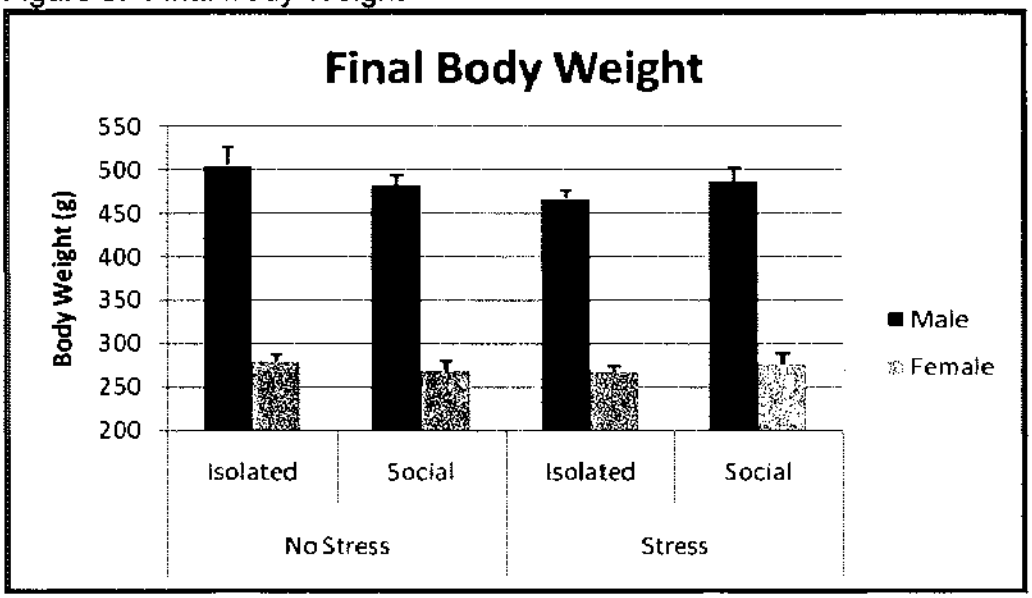
more weight than stress rats over time; and a time x sex interaction (F [3, 213] = 252.6, p < 0.01) with male rats gaining more weight than females over time.

Figure 8. Body Weight



Body weight also was analyzed on the final day of the experiment. There was a significant main effect for sex, such that males weighed more than females (F [1, 70] = 784.29, p < 0.01) (see Table 10 and Figure 9). There also was a trend toward a stress x housing interaction (F [1, 70] = 2.83, p = 0.09) with offspring from the prenatal stress and social housing conditions weighing the most (StS* > NSI > NSS > StI*). There was no main effect for stress or housing and an internal analysis of sex revealed that males were responsible for the stress main effect as there was no main effect of stress for the females.

Figure 9. Final Body Weight



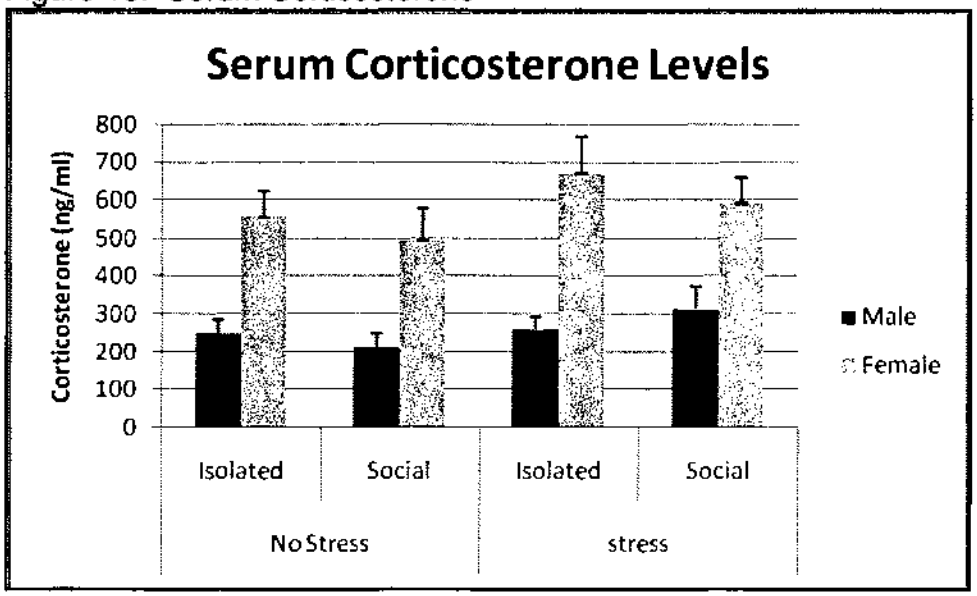
Summary of Body Weight Data. At weaning, offspring (of both sexes) that had mothers that were stressed during pregnancy weighed less than offspring that had mothers not stressed during pregnancy. This stress difference persisted for males throughout the experiment (i.e., into adulthood), but dropped off for females as they matured. Male offspring gained more weight throughout the experiment than female offspring and males continued to weigh more at the end of the experiment.

Corticosterone

Corticosterone is a biochemical released by the hypothalamic-pituitary-adrenal (HPA) axis as a part of the stress response. Serum corticosterone was measured in the present experiment to determine if the prenatal stress had a lasting effect on offspring into adulthood and if differences in sex or prenatal enrichment modified the effect of prenatal stress.

An ANOVA for all subjects with a one-tailed test (because of the directional hypothesis) revealed a significant main effect for stress ($F [1, 70] = 3.32$, $p = 0.07$). Offspring whose mothers had been stressed months earlier while they were *in utero* (stress condition) had higher serum corticosterone values than did offspring of mothers who were not stressed during pregnancy (no-stress condition) (see Figure 10 and Tables 11 and 12). There also was a sex effect ($F [1, 70] = 51.99$, $p < 0.01$) in which females had higher serum corticosterone levels than males. There was no significant effect for housing or any significant interactions.

Figure 10. Serum Corticosterone

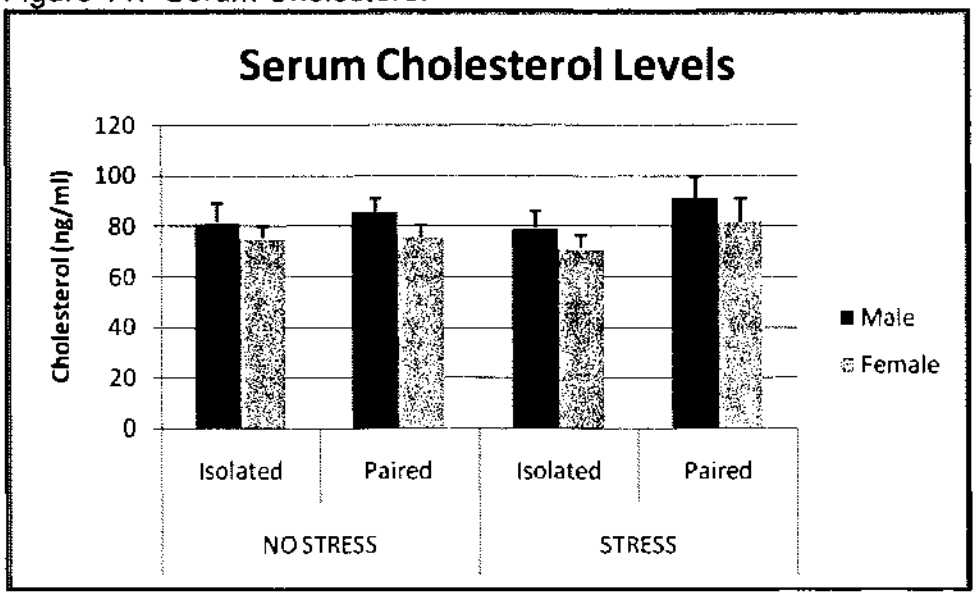


Summary of Corticosterone Data. Acute stress during pregnancy increased serum corticosterone in offspring – months after the stress was experienced. Females had higher levels of corticosterone compared to males. There was no effect of housing.

Cholesterol

Cholesterol is a lipid in the body's cells and blood. It was included in the present experiment because high levels can lead to atherosclerosis. Atherosclerosis is when the arteries become narrowed and blood flow to the heart muscle is slowed down or blocked which can lead to a heart attack. There were no significant main effects for stress, housing or sex (see Figure 11 and Tables 13 and 14).

Figure 11. Serum Cholesterol



Summary of Cholesterol Data. There were no effects of prenatal stress, prenatal enrichment, or sex on adult cholesterol levels.

C-Reactive Protein

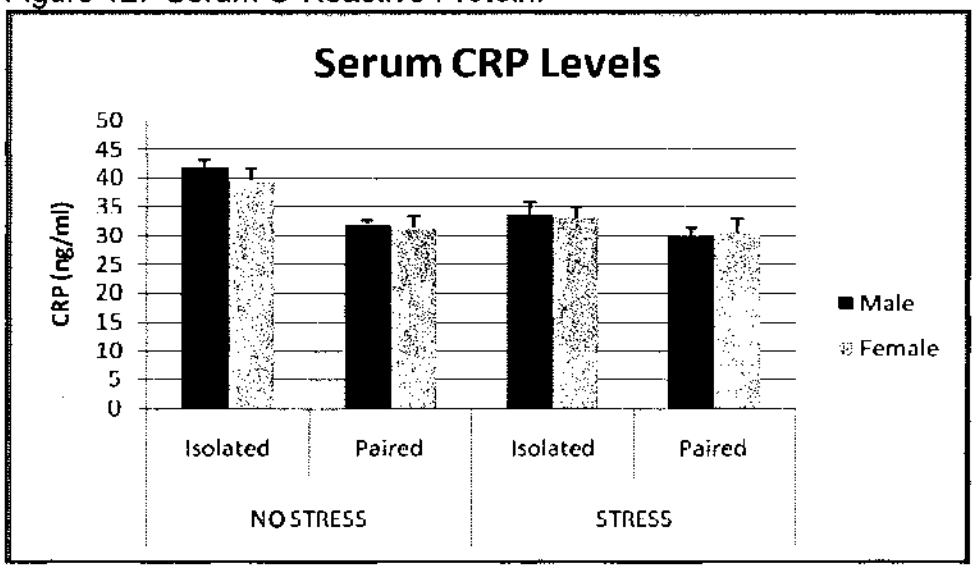
C-Reactive Protein (CRP) is a marker of inflammation relevant to physical and mental health. CRP levels consistently predict recurrent coronary events in patients with unstable angina and history of heart attack. Higher levels also are associated with lower survival rates in these patients (American Heart

Association, 2009). Major depression has been shown to be associated with activation of the inflammatory response, including increases in CRP (Danner, Kasl, Abramson, & Vaccarino, 2003) and antidepressants decrease CRP regardless of whether or not the depression remitted (O'Brien, Scott, & Dinan, 2006).

Non-stress animals had higher CRP levels than stress animals ($F [1, 71] = 11.21$, $p < 0.01$) (see Figure 12 and Tables 15 and 16). An internal analysis of sex revealed that the stress effect was mainly due to the males ($F [1, 37] = 8.45$, $p < 0.01$); females only had a trend toward a significant main effect of stress ($F [1, 37] = 2.96$, $p = 0.05$) (see Tables 17 and 18).

There also was a main effect for housing. Isolated animals had higher CRP than socially housed animals and this difference was true for both sexes ($F [1, 71] = 21.23$, $p < 0.01$). There was a stress x housing interaction ($F [1, 71] = 5.24$, $p < 0.05$); non-stress/isolated had the highest CRP values compared with all other groups (NSI* > StI* ≥ NSS ≥ StS).

Figure 12. Serum C-Reactive Protein.



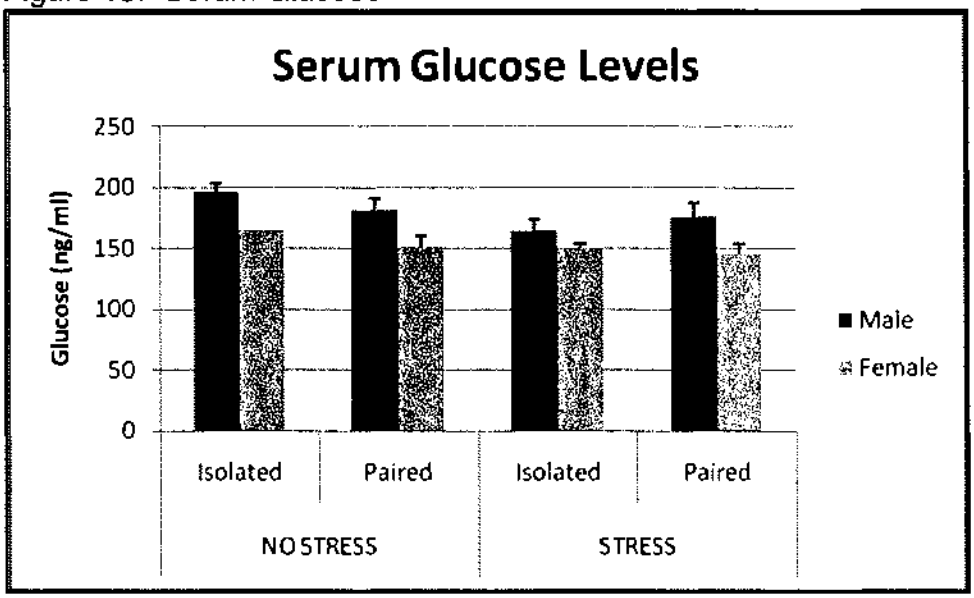
Summary of C-Reactive Protein. Prenatal stress as well as prenatal and weaning environment had a lasting effect on CRP in adult offspring. Specifically, offspring that had mothers stressed during pregnancy showed lower CRP levels than offspring that had mothers not stressed during pregnancy, but this difference was mostly the case for male offspring. Offspring that had mothers housed in isolation during gestation and weaning also had higher CRP levels than offspring that had mothers housed in social enrichment. Offspring that had non-stressed and isolated mothers showed the highest CRP levels compared with all other groups.

Serum glucose

Serum glucose is the assessment of the glucose (sugar from carbohydrates) in the blood. High blood sugar levels predict heart disease in patients with and without diabetes. In patients with diabetes, high serum glucose levels can lead to damage inside blood vessel walls. This damage makes it easier for fatty deposits (plaques) to form in arteries and cause narrowing or blockages that can lead to heart attacks or strokes. Serum glucose was included in the present experiment because it can contribute to heart disease risk.

Non-stress rats had higher glucose levels than stress rats ($F [1, 72] = 5.29$, $p < 0.05$). In addition, male rats had higher glucose levels than female rats ($F [1, 72] = 17.46$, $p < 0.01$) (see Figure 13 and Tables 19 and 20). An internal analysis of sex revealed that males were mostly responsible for the stress effect ($F [1, 37] = 3.27$, $p = 0.08$) as there were no significant effects for the females because of small effect sizes (see Table 21).

Figure 13. Serum Glucose



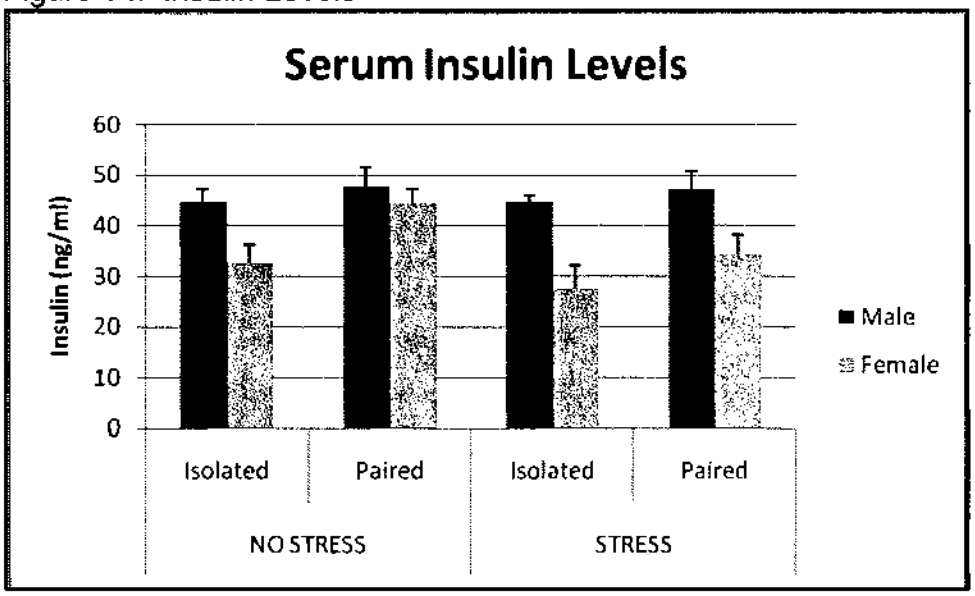
Summary of Glucose Data. Offspring whose mothers were stressed during pregnancy had lower serum glucose values *as adults* than offspring whose mothers were not stressed during pregnancy. This effect was mostly apparent in male offspring. Male offspring also had higher serum glucose levels than female offspring regardless of stress or housing condition.

Insulin

Insulin is a hormone produced by the pancreas that allows glucose to enter liver and muscle cells. It was included in the present experiment because insulin resistance (a condition in which the body does not produce insulin or does not use it effectively) combined with obesity and elevated lipids increases the risk of cardiovascular disease. High insulin levels *per se* also cause the retention of sodium, which causes fluid retention, and can lead to high blood pressure and congestive heart failure (Gallistl, Sudi, Mangge, Erwa, & Borkenstein, 2000).

Males had higher insulin levels compared with females ($F [1, 68] = 22.11$, $p < 0.01$). Socially housed rats had higher insulin levels compared with isolated animals ($F [1, 68] = 6.49$, $p < 0.05$) (see Figure 14 and Tables 22 and 23). There were no significant interactions. An internal analysis of sex revealed that the stress ($F [1, 30] = 3.63$, $p = 0.07$) and housing ($F [1, 30] = 5.77$, $p < 0.05$) effects were mainly due to the males as the females did not show any significant main effects or interactions (see Table 24).

Figure 14. Insulin Levels



Summary of Insulin Data. Male offspring had higher levels of insulin compared to female offspring. The housing environment during prenatal and weaning time periods affected insulin levels, but only for males. Male offspring from a social environment had higher insulin levels compared with male offspring from an isolated environment. There were no effects for females and no prenatal stress effects on insulin levels.

Heart Morphology

Heart morphology includes the assessment of the heart's dimensions (e.g., left ventricular size, length, width, weight). Heart morphology was included in the present experiment because changes in the heart structure occur in various cardiovascular conditions.

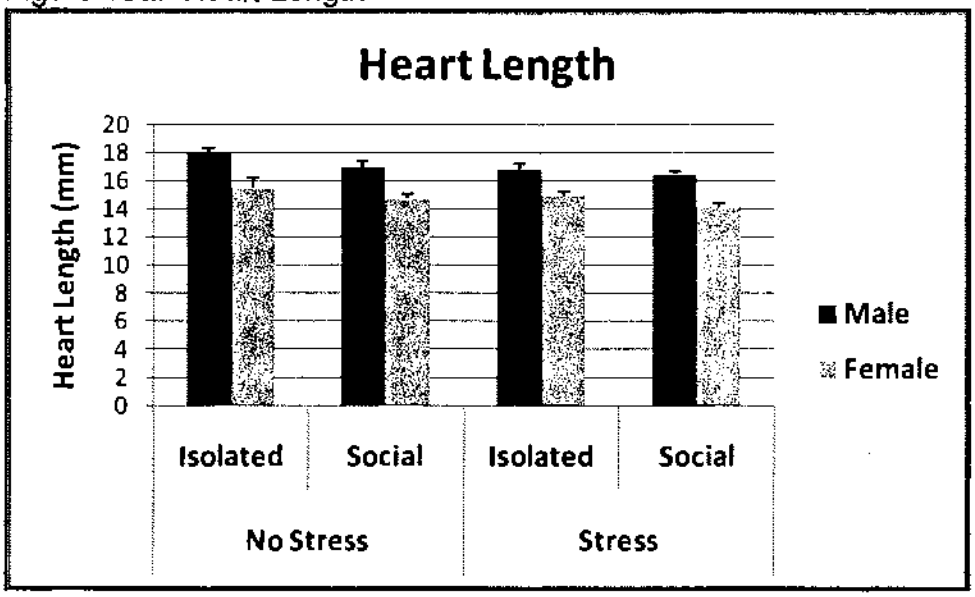
A two-way between-groups multivariate analysis of covariance with final body weight as the covariate was performed to investigate the effects of sex, stress, and housing conditions on various heart measures. A MANCOVA was performed because these heart measures are conceptually and statistically correlated. Body weight was used as a covariate because it was significantly correlated with each of the heart measurements. The heart measures included: heart length, weight, width; left and right ventricle width (LV and RV, respectively); and septal, lateral, posterior and anterior wall widths (see Tables 25a-b, 26a-b, and Figures 15a-15i).

Preliminary assumption tests of homogeneity revealed no violation of Box's test, but there was a violation of homogeneity on Levene's Test for the measures of heart weight and LV. To correct this violation, a more conservative alpha of 0.025 was used for these variables when considering the between-subjects effects only (Tabachnik & Fidell, 2007). There were significant main effects of prenatal stress ($F [9, 62] = 2.36$, $p = 0.02$) and housing on the combined heart variables ($F [9, 62] = 4.26$, $p < 0.01$). There also was a trend for a main effect of sex ($F [9, 62] = 1.90$, $p = 0.07$) on the combined heart variables using body weight as a covariate. There were no significant interactions.

When the results for the dependent variables were considered separately, there were effects of prenatal stress, housing, or sex on all heart measures. Table C (below) summarizes the significant heart findings.

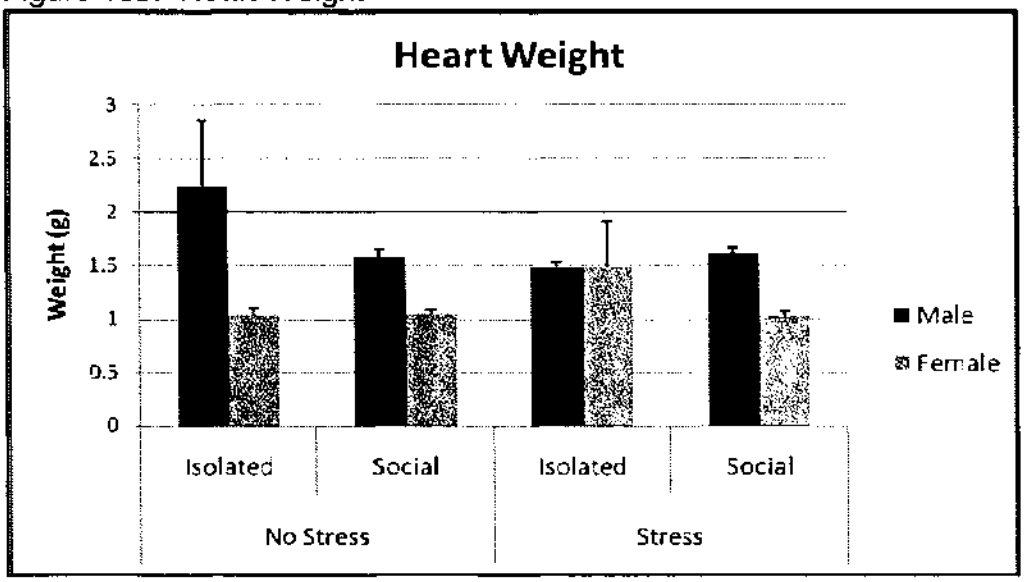
Heart length. Non-stress rats had longer hearts than stress rats ($F [1, 70] = 8.94$, $p < 0.01$) and this was true for both sexes. Isolated rats had longer hearts than social rats ($F [1, 70] = 13.20$, $p < 0.01$) which was also true for both sexes. There were no sex main effects or any significant interactions..

Figure 15a. Heart Length



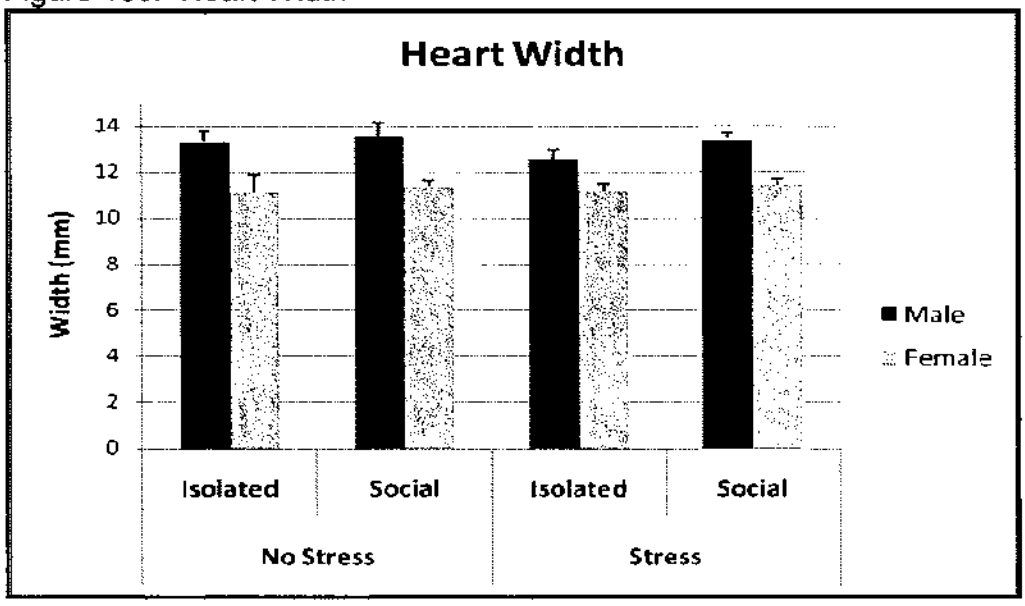
Heart Weight. There was no effect of stress or housing on heart weight. There was a trend toward a sex ($F [1, 70] = 3.66$, $p = 0.06$) effect on heart weight such that males had heavier hearts than females even when accounting for body weight.

Figure 15b. Heart Weight



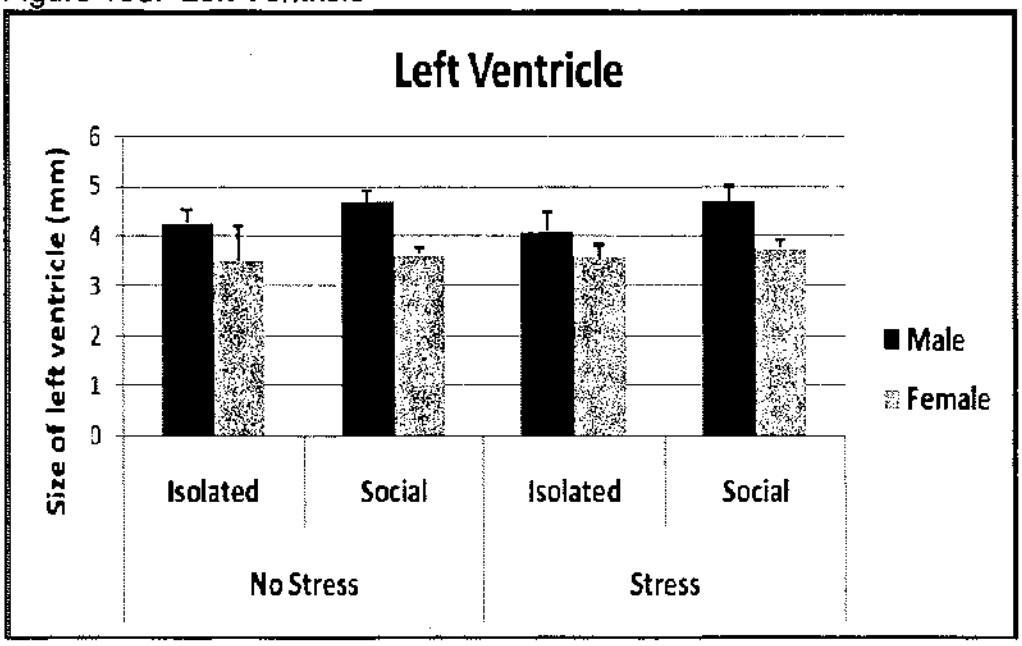
Heart Width. There was no effect of stress on heart width. There was an effect of housing on heart width ($F [1, 70] = 4.29$, $p < 0.05$) such that isolated rats had narrower widths than social rats. An internal analysis revealed that this housing effect was mostly due to the males ($F [1, 36] = 3.23$, $p = 0.08$); females had no stress effects on heart width. There was no sex main effect or any interactions.

Figure 15c. Heart Width



Left Ventricle. There was no stress effect on left ventricle size. There was a trend toward a housing effect (F [1, 70] = 3.59, p = 0.06) such that isolated rats had smaller left ventricle's compared with social rats and an internal analysis of sex revealed that this effect was mostly due to males (F [1, 36] = 3.29, p = 0.08) with no effects for females on left ventricle. There was no sex main effect or any significant interactions.

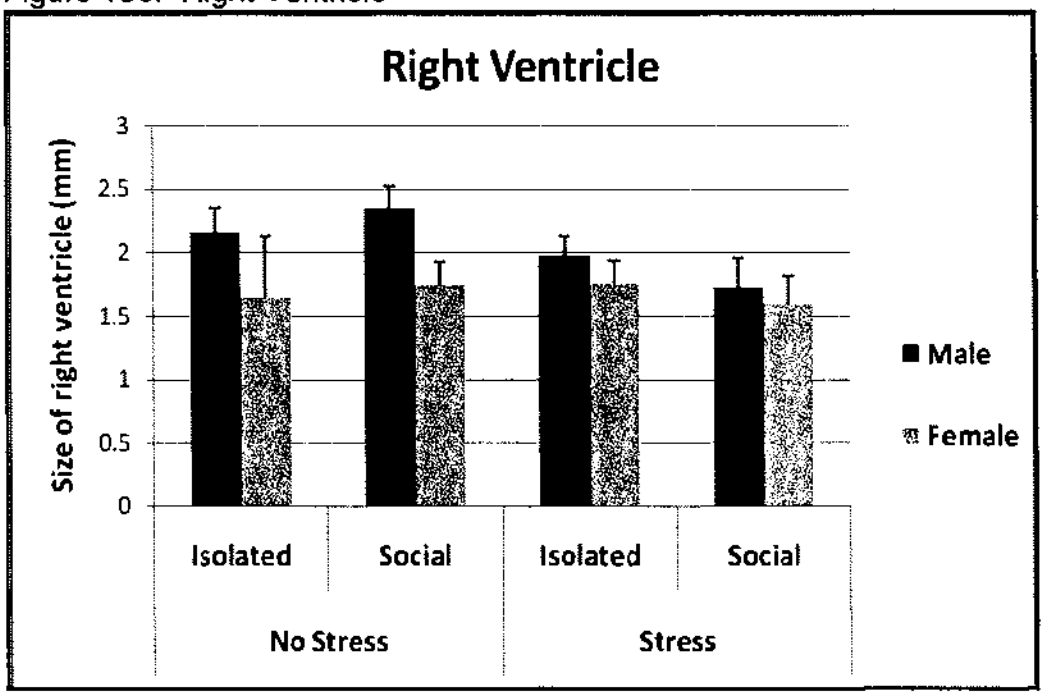
Figure 15d. Left Ventricle



Right Ventricle. There were no overall stress or housing effects on right ventricle size. An internal analysis of sex revealed that males had a trend toward a stress effect with stressed rats having narrower right ventricles (F [1, 36] = 2.93, p = 0.10). There was no stress effect for females. There was a trend toward a sex effect (F [1, 70] = 3.38, p < 0.05) such that males had wider right ventricles compared with females. There also was a trend toward a significant stress x housing interaction (F [1, 70] = 3.74, p = 0.06) such that stress/social rats had the smallest right ventricles compared with all other groups and an

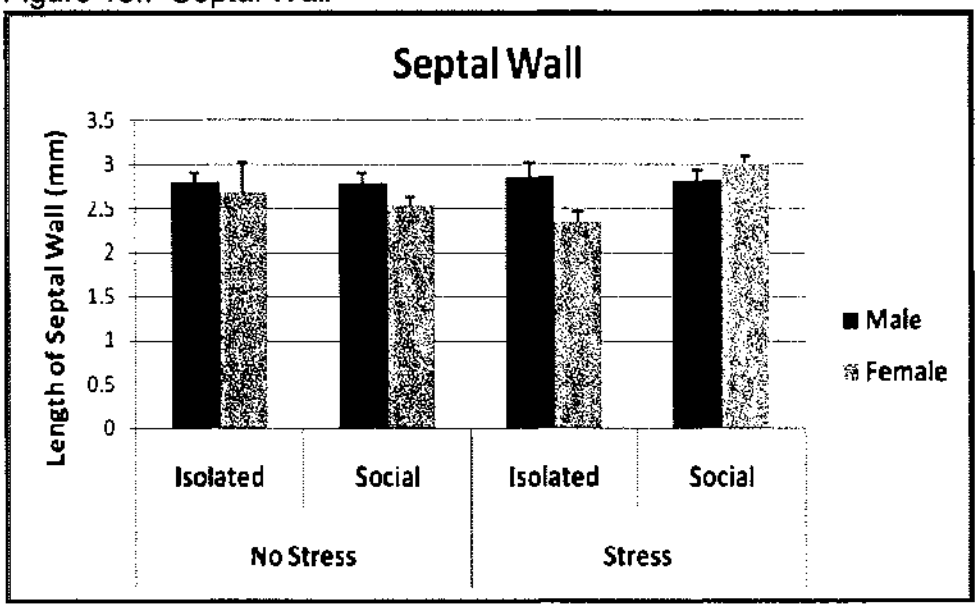
internal analyses revealed that this interaction was mostly due to males (F [1, 36] = 2.89, p = 0.10); females did not have any effects on right ventricle.

Figure 15e. Right Ventricle



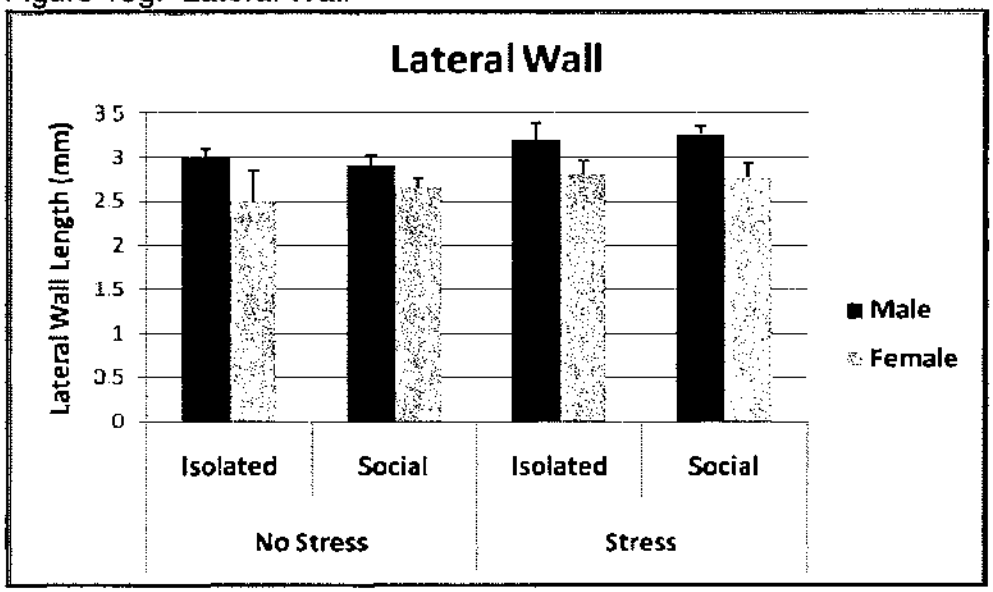
Septal Wall. There were no overall stress, housing, or sex main effects on septal wall. An internal analysis of sex revealed that housing impacted the septal wall for females only with social rats having thicker septal walls than isolated rats (F [1, 33] = 6.12, p < 0.05). There was a trend toward a significant stress x housing interaction (F [1, 70] = 2.89, p = 0.09) such that the stress/social rats had the thickest septal wall (see Figure 15f). However, an internal analysis revealed that this interaction was only true for females (F [1, 33] = 13.68, p < 0.01). There also was a trend toward a sex x housing interaction (F [1, 70] = 2.89, p = 0.09) such that isolated/female rats had the thinnest septal walls.

Figure 15f. Septal Wall



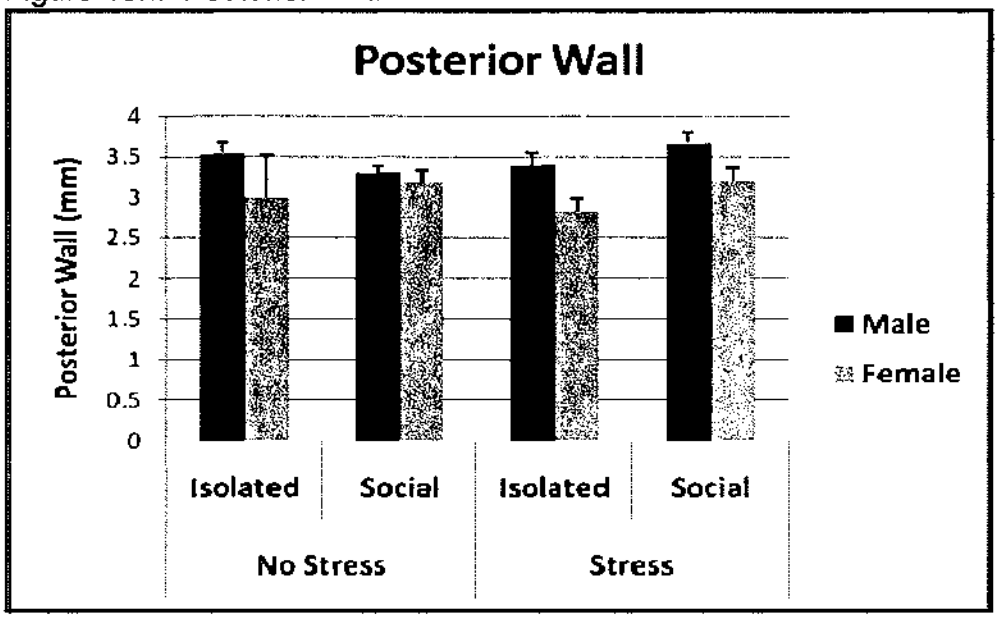
Lateral Wall. Stress rats had thicker lateral walls than non-stress rats ($F [1, 70] = 7.40$, $p < 0.01$) but an internal analysis revealed that this effect was only true for males ($F [1, 36] = 5.74$, $p < 0.05$). There was no housing effect but there was a sex effect ($F [1, 70] = 5.26$, $p < 0.05$) such that males had thicker lateral walls than females. There were no significant interactions.

Figure 15g. Lateral Wall



Posterior Wall. There were no overall effects of prenatal stress, housing, or sex for posterior wall measurements. An internal analysis revealed that females had a trend toward a main effect of housing ($F [1, 38] = 3.81$, $p < 0.06$) such that social females had thicker posterior walls than isolated females.

Figure 15h. Posterior Wall



Anterior Wall. There were no main effects for anterior length. There was a trend for stress x housing ($F [1, 70] = 4.15$, $p < 0.05$) such that no-stress/social rats had the shortest anterior walls.

Figure 15i. Anterior Wall

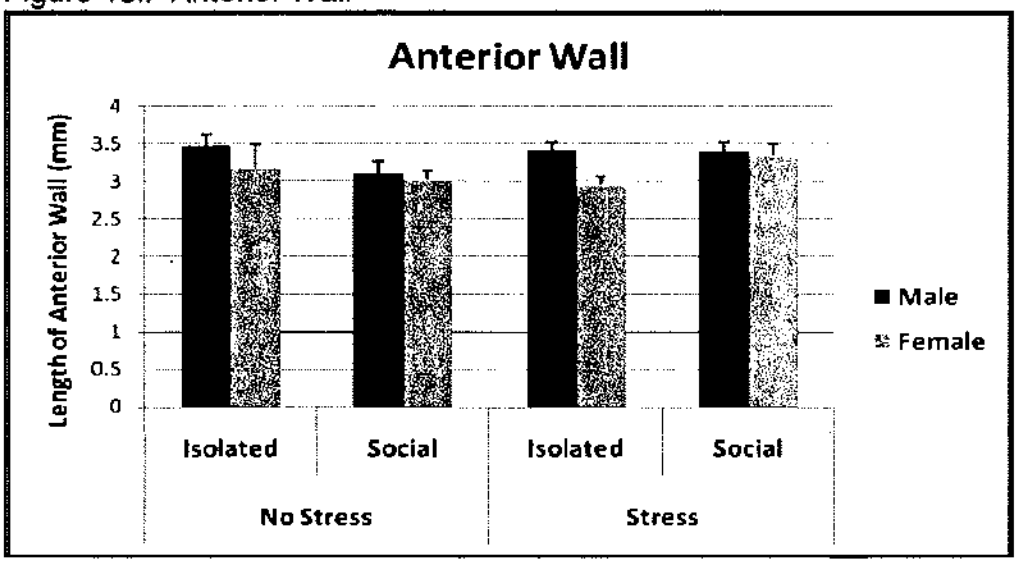


Table C. Summary of Heart Morphology Findings

Heart Measure-ment	Stress Main Effect			Housing Main Effect			Sex Main Effect	Interaction			Direction of Interaction
	Overall	♂	♀	Overall	♂	♀		Overall	♂	♀	
Length	NS > St	NS > St	NS > St	I > S	I > S	I > S	-----	-----	-----	-----	-----
Weight	-----	-----	-----	-----	-----	-----	M > F^	-----	-----	-----	-----
Width	-----	-----	-----	I < S^	I < S^	-----	-----	-----	-----	-----	-----
LV	-----	-----	-----	I < S^	I < S^	-----	-----	-----	-----	-----	-----
RV	-----	NS > St^	-----	-----	-----	-----	M > F^	Stress x housing^	Stress x housing^	-----	NSS largest; StS smallest
Septal	-----	-----	-----	-----	-----	I < S	-----	Stress x housing^	-----	Stress x housing^	StS largest; NSS smallest
Lateral	NS < St	NS < St	-----	-----	-----	-----	M > F	-----	-----	-----	-----
Posterior	-----	-----	-----	-----	-----	I < S^	-----	-----	-----	-----	-----
Anterior	-----	-----	-----	-----	-----	-----	-----	Stress x housing^	-----	-----	NSS shortest

^ indicates a trend toward significance ($p \leq 0.10$)

Summary of Heart Morphology. Prenatal stress, housing environment during gestation and weaning, and sex all had effects on the offspring's adult heart. For both sexes, adult offspring of mothers that had been stressed had

shorter heart lengths and adult offspring of mothers that were housed socially during pregnancy and weaning had shorter heart lengths.

The sex of the offspring affected the heart despite statistically accounting for body weight. Female hearts also weighed less and had smaller right ventricles and lateral walls compared with male offspring. Male offspring that were prenatally stressed had thicker lateral walls than males not prenatally stressed, but this relationship did not exist for females. Female offspring of mothers that were socially housed had thicker septal and posterior walls than females not prenatally stressed but this relationship did not exist for males.

There were significant interactions on some heart dimensions, but consistent patterns were difficult to detect. It did appear that hearts from offspring that were not prenatally stressed and were exposed to a prenatal/weaning early social environment were affected most by these early experiences (see Table B).

Summary of Biological Variables

To summarize the biological variables, the major findings are discussed in this section. In addition, two tables are presented. The first table (Table D) at the end of this section, presents the major biological findings. The second table (Table 75a) in the appendix, summarizes all of the biological findings.

Prenatal stress, prenatal and weaning housing condition, and sex of the offspring had significant effects in later adulthood. **Prenatal stress:** (1) **decreased body weight** for both sexes at the time of weaning and for the male offspring throughout the experiment and into adulthood; (2) **increased**

corticosterone levels in offspring *as adults* compared with adult offspring that had non-stressed mothers during pregnancy; (3) prenatal stress also **decreased CRP** in both sexes as adults and **serum glucose** values in adult male offspring; (4) **affected heart morphology** in some ways that are consistent with rats that had been stressed in late adolescence or adulthood (Elliott et al., 2003).

Prenatal social enrichment (pair housing) and weaning social enrichment resulted in: (1) **lower CRP** in adulthood for both sexes; (2) **higher insulin** in adulthood for males; (3) **shorter heart lengths** in adulthood (for both sexes) and **thicker septal and posterior walls** for females.

Males had significant effects between prenatal groups on body weight, CRP, and **heart variables**. **Females**, in contrast, only had significant differences between prenatal groups on **heart morphology**. Therefore, **males may be more vulnerable to the biological effects** of prenatal stress. There also were stress x housing interactions however consistent patterns were difficult to detect.

Table D. Summary of Significant Biological Findings

Variable	Stress Main Effect			Housing Main Effect			Sex Main Effect	Interaction			Direction of Interaction
	Overall	♂	♀	Overall	♂	♀		Overall	♂	♀	
BW	NS > St	NS > St	-----	I > S	I > S	I > S	M > F	-----	-----	-----	-----
Cort	NS < St	-----	-----	-----	-----	-----	M > F	-----	-----	-----	-----
CRP	NS > St	NS > St	-----	I > S	I > S	I > S	-----	Stress x housing	-----	-----	NSI>StI> NSS>StS
Glucose	NS > St	-----	-----	-----	-----	-----	M > F	-----	-----	-----	-----
Insulin	-----	-----	-----	I < S	I < S	-----	M > F	-----	-----	-----	-----

Note: for a summary of Heart Morphology findings see Table C

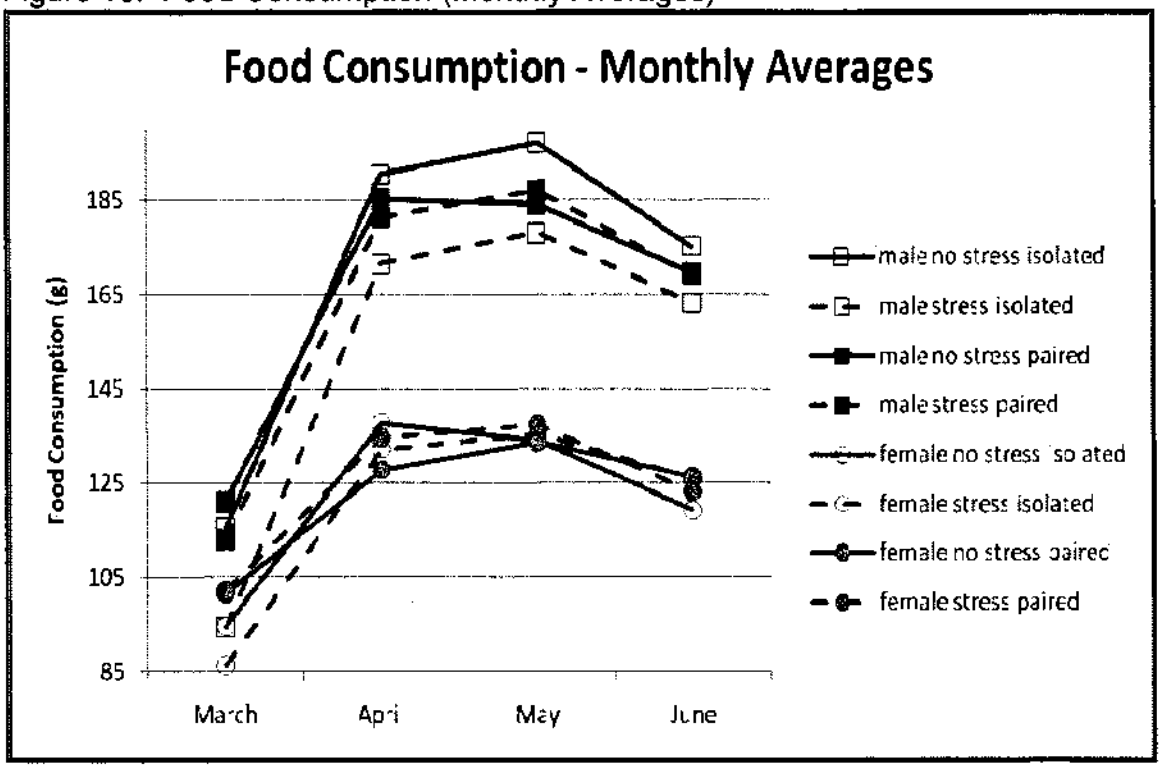
Behavioral Variables

Food Consumption

Males consumed more food than females ($F [1, 58] = 175.29$, $p < 0.01$ (see Figure 16 and Tables 27, 28a-b). An internal analysis of sex revealed that prenatally stressed males ate less food than non-prenatally stressed males ($F [1, 34] = 6.95$, $p < 0.05$) but there was no stress effect for females (see Table 29). There also was a significant sex x stress interaction ($F [1, 58] = 5.99$, $p < 0.05$) such that non-stress males ate the most food compared with all other groups (NSM* > StM > StF > NSF*) [groups with a superscript asterisk * are different from each other].

Food consumption increased over time for both sexes ($F [3, 174] = 303.33$, $p < 0.01$). There was a significant interaction of time x housing ($F [3, 174] = 2.95$, $p < 0.05$) such that social rats consumed more food compared to isolated rats over time for male offspring only. There also was a time x sex ($F [3, 174] = 25.79$, $p < 0.01$) which revealed that males consumed more food throughout the experiment compared with females. There were no main effects for stress or housing.

Figure 16. Food Consumption (Monthly Averages)



Summary of Food Consumption. Male offspring ate more than females and all conditions increased food consumption over time. Non-stress, isolated males consumed the most food compared with all other groups.

Open Field

Open field locomotion refers to an animal's behavior when placed in a non-home cage arena. Locomotor activity can be an index of an animal's general health and activity and time spent in the center of the chamber can be an index of anxiety. Horizontal activity was included in the present experiment to index general arousal and activity and to determine if overall movement may be a factor to consider for the other behavioral variables that included movement. Center time was used to index anxiety.

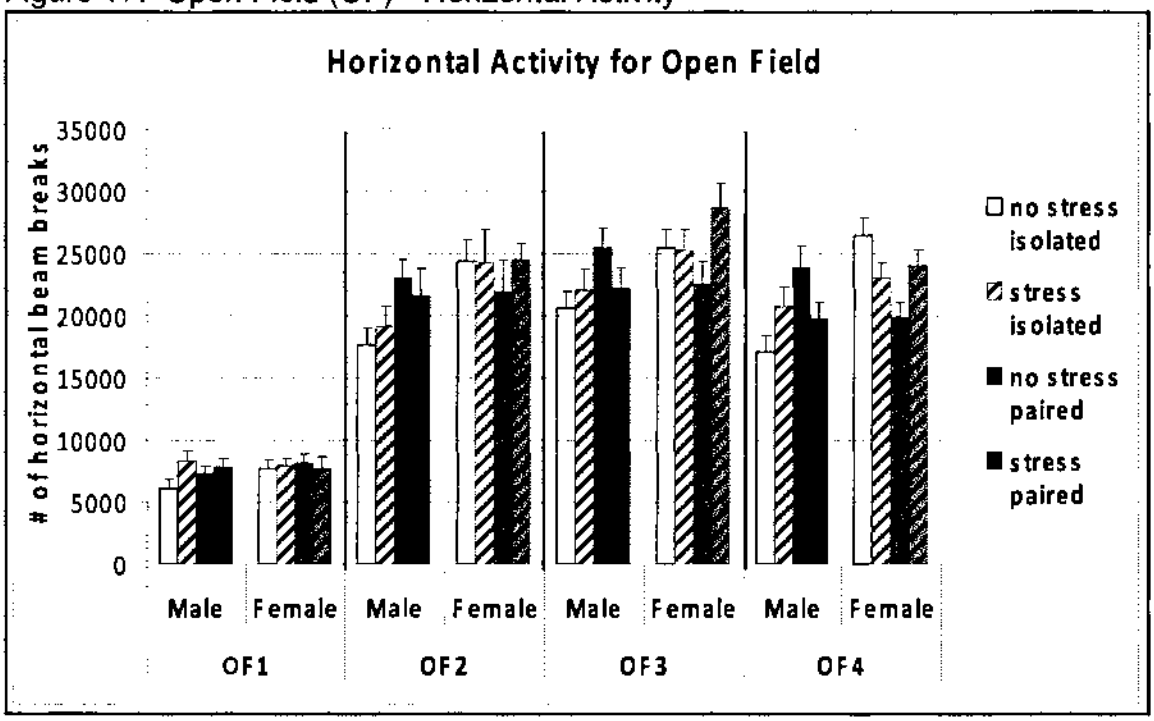
A repeated-measures ANOVA was conducted on horizontal activity across four time periods (one measurement each month). All animals increased their activity over time ($F [1, 71] = 277.64$, $p < 0.01$). Female offspring had greater activity than did male offspring ($F [1, 71] = 10.52$, $p < 0.01$) (see Figure 17 and Tables 30 and 31a-b). There also was a housing x sex interaction ($F [1, 71] = 4.79$, $p < 0.05$) with isolated male rats having the least activity (IF* > SF > SM > IM*) and a stress x housing x sex interaction ($F [1, 71] = 7.69$, $p < 0.01$) with non-stress/isolated male rats having the least amount of activity and stressed, social females having the most (StSF* > NSIF > StIF > NSSM > NSSF > StSM > StIM > NSIM*).

An internal analysis of sex was conducted and revealed that males had a main effect for housing ($F [1, 37] = 5.52$, $p < 0.05$) with offspring from prenatal and weaning social environments having more activity than males from isolated environments. There also was a stress x housing interaction ($F [1, 37] = 4.41$, $p < 0.05$) with non-stress social rats having the most horizontal activity (NSS* > StS = StI > NSI*) (see Tables 32 and 33). Females had a trend toward a stress x housing interaction ($F [1, 34] = 3.34$, $p = 0.08$) with non-stressed, social rats having the least activity (StS* = NSI = StI > NSS*).

In summary of horizontal activity in the open field, male offspring had less activity than females. There were several interactions indicating that isolated males had low amounts of horizontal activity. There was a housing effect on horizontal activity for males only such that social males had more activity than

isolated males. There was no effect of prenatal stress or prenatal or weaning housing environment on horizontal activity.

Figure 17. Open Field (OF) - Horizontal Activity



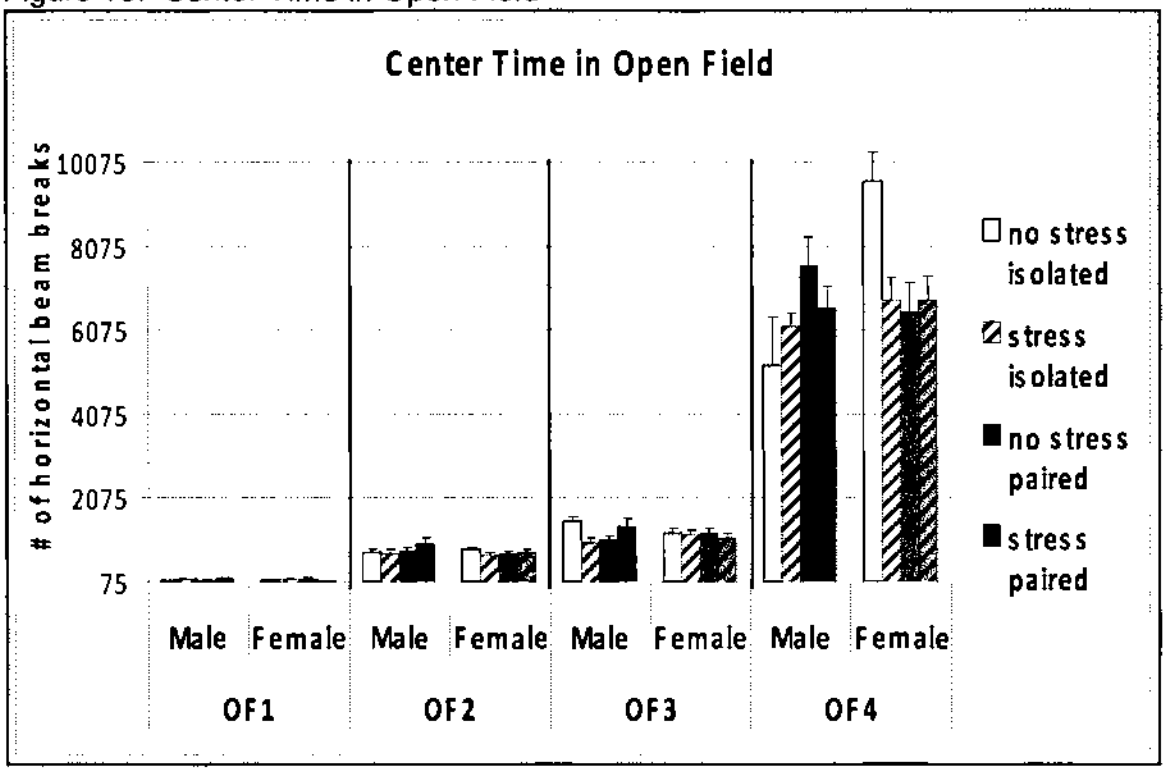
For center time, separate ANOVAs at each open field measure were conducted to determine anxiety levels at different points throughout the experiment (see Tables 34-41). Open Field #1 was conducted just after weaning on postnatal (PN) days 25 and 26. There were no significant main effects or interactions for center time (see Figure 18). Open Field #2 was conducted during adolescence on PN Days 48 and 49. There were no significant main effects or interactions. Open Field #3 was conducted in early adulthood on PN Days 84 and 85 and right after the forced swim test. There were no significant main effects but there was a trend toward a stress x housing interaction ($F [1, 71] = 3.65$, $p = 0.06$), such that non-stress isolated rats had the most center time (therefore the least anxiety) and stressed isolated had the least center time

(therefore the most anxiety) compared with all other groups (NSI* > StS > NSS > StI*). There also was a significant stress x housing x sex interaction with non-stress/isolated male rats showing the most center time and stressed/isolated male rats showing the least center time (therefore the most anxiety-like behavior) (NSIM* > StSM **> SNIF > NSSF > StIF > StSF > NSSM* > StIM**). An internal analysis of sex revealed that only males had a significant stress x housing interaction ($F [1, 37] = 7.24$, $p < 0.01$) (NSI* > StS > NSS* = StI*).

Open Field #4 occurred just before the end of the experiment on PN Days 128 and 129. Female offspring had greater center time compared with male offspring ($F [1, 71] = 4.10$, $p < 0.05$) and an internal analysis of sex revealed a main effect of housing for both sexes but in opposite directions. Isolated males had less center time (i.e., more anxiety-like behavior) than social males ($F [1, 37] = 4.56$, $p < 0.05$). Isolated females had greater center time (i.e., less anxiety-like behavior) compared with social females ($F [1, 34] = 4.10$, $p = 0.05$). There was a housing x sex interaction ($F [1, 71] = 8.65$, $p < 0.01$) such that isolated females had the most center time and isolated males had the least amount of center time compared with all other groups (IF* > SM > SF* > IM*). The internal analysis of sex revealed that there was a stress x housing interaction for the females only ($F [1, 34] = 4.10$, $p = 0.05$) (NSI* > StS* = StI* > NSS*). This interaction partially explains why there also was a three way, stress x housing x sex, interaction ($F [1, 71] = 6.26$, $p < 0.05$) such that non-stress/social females had the most amount of center time and stress/isolated male rats had the least amount of center time

(NSIF* > NSSM> StIF > StSF > NSSF* > StSM > StIM > NSIM). There were no stress or housing main effects.

Figure 18. Center Time in Open Field

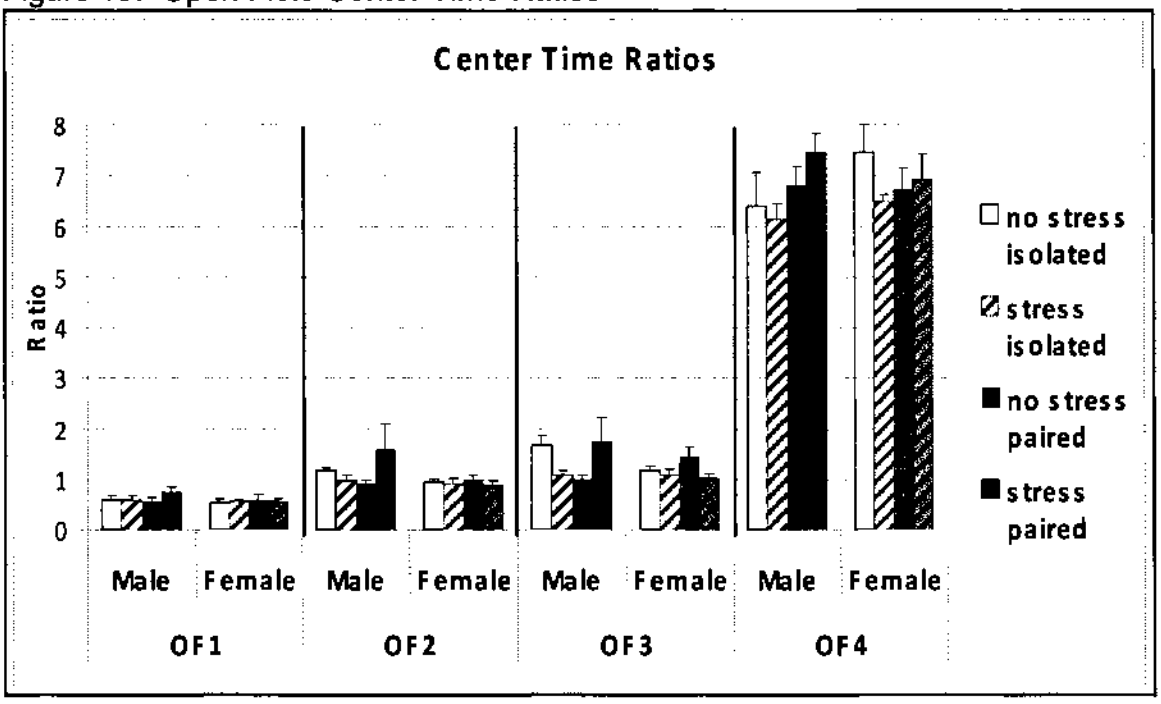


Center time also was analyzed in the context of overall horizontal activity because there were differences in horizontal activity among the groups. A center time ratio was created by dividing center time by overall movement time in the chamber. These ratios were analyzed using separate ANOVAs for each time point (see Figure 19 and Tables 42-49).

For the Open Field #1 ratio, an internal analysis of sex revealed a trend toward a stress main effect with non stress males having greater center time ratios (i.e., less anxiety-like behavior) than stressed male offspring ($F [1, 37] = 2.86$, $p = 0.09$). There were no other significant main effects or interactions for Open Field #1. There were no significant main effects or interactions for center

time ratios in Open Field #2. For ratios in Open Field #3, there were no significant main effects. There was a stress x housing x sex interaction such that stress/social male rats had the most amount of center time and stress/social female rats and non-stress/social male rats had the least (StSM* > NSIM > NSSF > NSIF > StIM = StIF > StSF > NSSM*). An internal analysis of sex revealed a trend toward a stress x housing interaction for males only ($F [1, 37] = 3.51$, $p = 0.07$) showing that stress, isolated male offspring had the most center time (an index of less anxiety-like behavior) compared with all other male conditions (StI* > NSS > NSI* > StS*). For ratios in Open Field #4, there were no significant main effects. There was a trend toward a stress x housing interaction ($F [1, 71] = 3.44$, $p = 0.07$), such that non-stress/isolated rats had the most center time and non-stress/social rats had the least (NSI* > StS > StI* > NSS). An internal analysis of sex revealed that females were responsible or this interaction and that female non-stress isolated offspring had the least amount of anxiety- like behavior ($F [1, 34] = 4.53$, $p < 0.05$) (NSI* > StS* > StI* > NSS*).

Figure 19. Open Field Center Time Ratios



Summary of Open Field. All animals increased horizontal activity over time. Female offspring had greater horizontal activity compared with male offspring. Male offspring that had mothers housed socially were more active than male offspring that had isolated mothers. There was no effect of prenatal stress on horizontal activity.

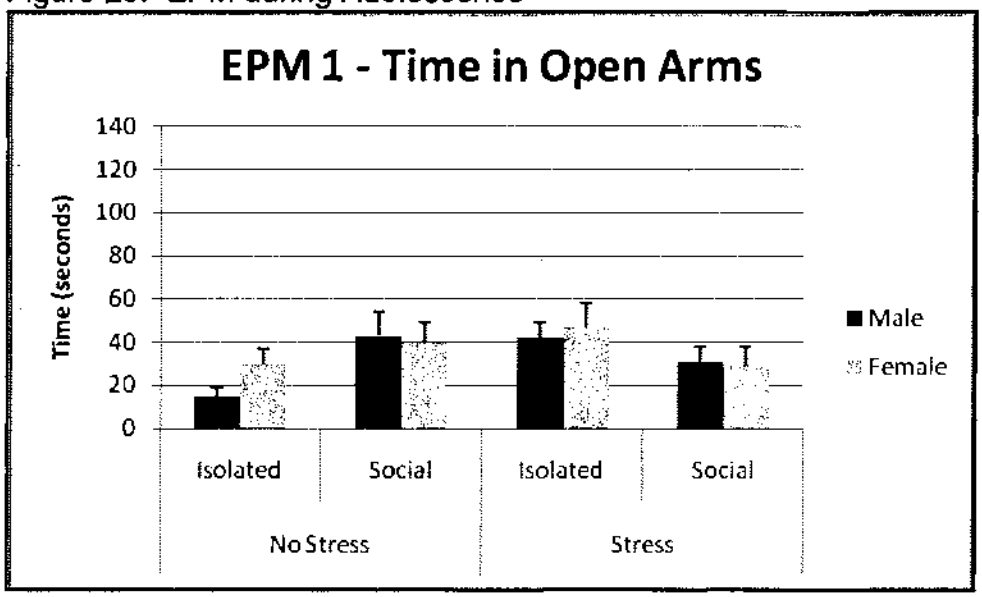
For center time, female adult offspring displayed less anxiety-like behavior than male adult offspring. Housing also influenced center time for adult offspring but in different directions for each sex. Males that had socially housed mothers had greater anxiety than males from isolated mothers. Females that had socially housed mothers had less anxiety-like behavior than females from isolated mothers. There was no effect of prenatal stress on center time or any consistent interactions.

Elevated Plus Maze

Elevated Plus Maze (EPM) is an index of anxiety such that time in the open arms is considered to reflect less anxiety-like behavior and time in the closed-arms is viewed as more anxiety-like behavior. EPM was assessed two times during the experiment, once at the beginning (during early adolescence) and once toward the end (during adulthood).

For the first EPM assessment (during adolescence), there were no significant main effects (see Figure 20). There was a significant stress x housing interaction ($F [1, 69] = 6.60$, $p < 0.05$) (see Tables 50 - 52). An internal analysis of sex revealed that only males had a stress x housing interaction ($F [1, 37] = 4.92$, $p < 0.05$) with non-stress isolated males showing the least amount of time in the open areas (an index of greater anxiety-like behavior) (NSS* > StI > StS > NSI*).

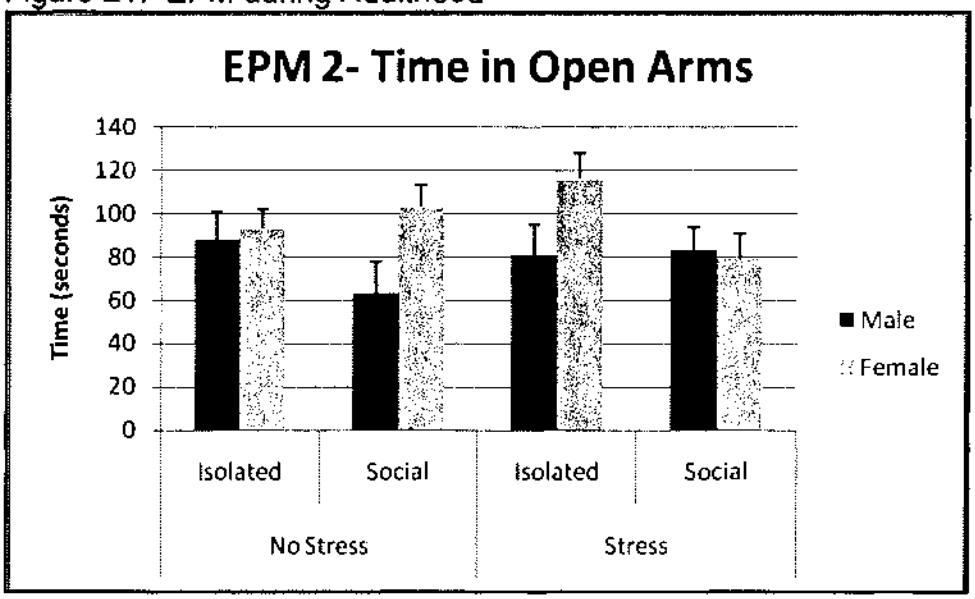
Figure 20. EPM during Adolescence



For the second EPM assessment (during adulthood), an ANOVA revealed no overall stress or housing main effects. There was a main effect for sex ($F [1,$

66] = 5.18, $p < 0.05$) such that females spent more time in the open arms (an index of less anxiety-like behavior) than males did (see Figure 21 and Tables 53 and 54). There also was a significant sex x stress x housing interaction (F [1, 66] = 4.79, $p < 0.05$) with stress/isolated female rats spending the most amount of time in the open arms (an index of less anxiety-like behavior) and non-stress/social male rats spending the least amount of time in the open arms (an index of greater anxiety-like behavior) (StIF* > NSSF** > NSIF > NSIM > StSM > StIM* > StSF > NSSM**). An internal analysis of sex revealed that only females had a stress x housing interaction with stress isolated fernales displaying the least anxiety-like behavior (F [1, 33] = 5.03, $p < 0.05$) (StI* > NSS* > NSI* > StS*).

Figure 21. EPM during Adulthood



Summary of EPM. During early adolescence, there was no overall effect of prenatal stress, prenatal or weaning housing, or sex. There was a significant stress x housing interaction for males only such that offspring of dams that were

prenatally stressed and isolated spent the most amount of time in the open arms. In adulthood, the patterns changed. Females clearly showed less anxiety-like behavior than males. Moreover, females of dams that were stressed and isolated showed the least amount of anxiety-like behavior compared with all other females.

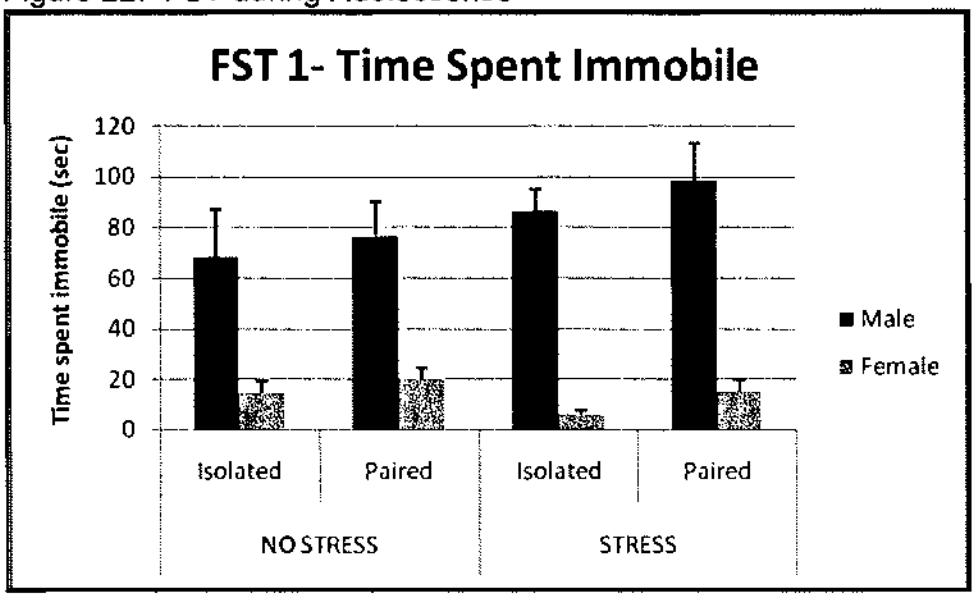
Forced Swim Test

Forced Swim Test (FST) provides a measure of depressive-like behavior. The more time spent immobile on day two of the assessment, the more an animal is considered to be engaging in learned helplessness, an index of depressive behavior.

On the first assessment of FST when the offspring were adolescents, there was no main effect of prenatal stress or sex (see Figure 22 and Tables 55 - 58). There was a significant main effect of housing ($F [1, 64] = 9.95$, $p < 0.05$) such that social rats had greater time spent immobile (an index of greater depression) compared to isolated rats. An internal analysis of sex revealed that this housing difference was significant for male offspring ($F [1, 33] = 5.34$, $p < 0.05$) and there was a trend toward significance for female offspring ($F [1, 31] = 2.97$, $p = 0.10$). There also was a trend for an overall stress x housing interaction ($F [1, 64] = 2.96$, $p = 0.09$). An internal analysis revealed that only the males had a stress x housing interaction trend ($F [1, 33] = 2.94$, $p = 0.10$) such that stress/social rats had the most immobile time (therefore the most depression) and stress/isolated had the least immobility (StS* > NSS > NSI >> StI*). There also was an overall sex x housing interaction ($F [1, 64] = 2.88$, $p = 0.09$) which

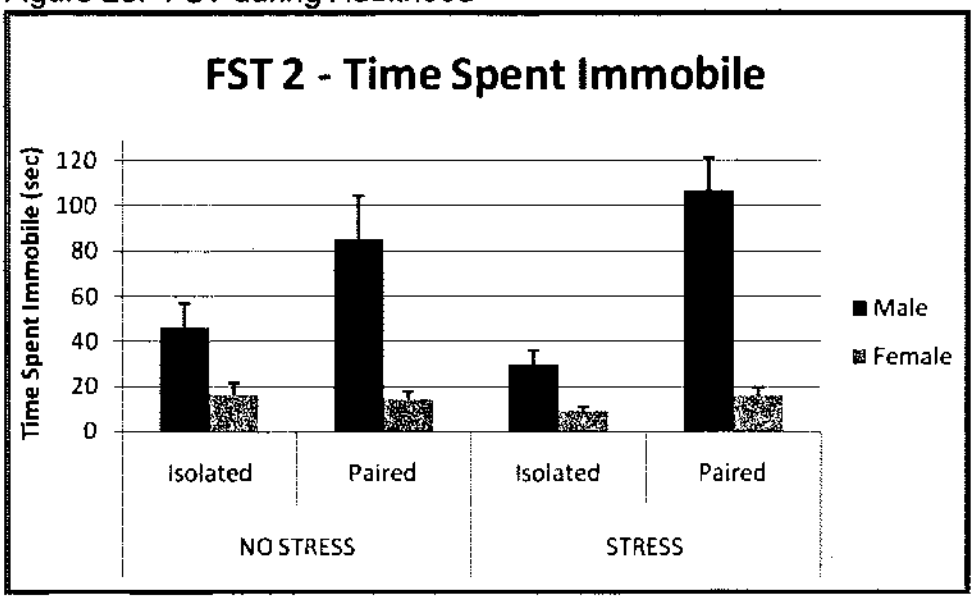
revealed that isolated females had the least amount of immobility and SM had the most compared with all other groups (SM* > IM* > SF > IF).

Figure 22. FST during Adolescence



The second assessment of FST occurred toward the end of the experiment when the offspring were adults. An ANOVA revealed no main effect of prenatal stress (see Figure 23 and Table 60). There was a significant main effect of housing ($F [1, 69] = 15.67$, $p < 0.01$) such that social animals continued to have more immobile time compared with isolated offspring. An internal analysis revealed that this effect only occurred for males ($F [1, 35] = 15.96$, $p < 0.05$). There also was an overall main effect of sex ($F [1, 69] = 51.12$, $p < 0.01$) such that males had more immobile time compared with females. There was an overall significant sex x housing interaction ($F [1, 69] = 13.33$, $p < 0.01$) such that social male rats spent the greatest amount of time immobile compared with all other groups (SM* > IM* > SF > IF) which is exactly the result found during the adolescent time period.

Figure 23. FST during Adulthood



Summary of FST. Male offspring showed more depressive-like behavior than female offspring and males that were offspring of socially housed mothers showed more depressive-like behavior than males whose mothers were prenatally isolated. There were no effects of prenatal stress on depressive-like behavior.

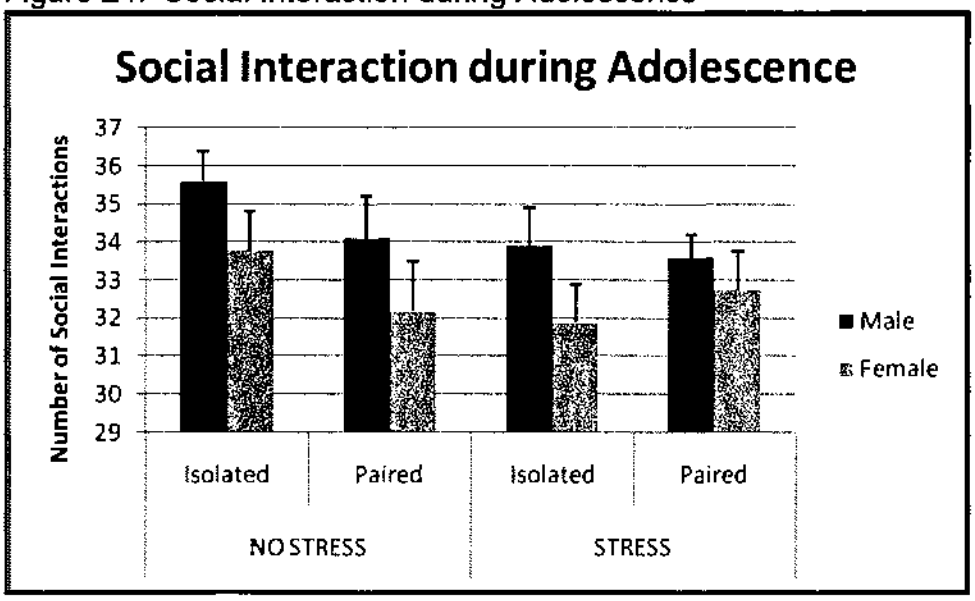
Social Interaction

Social interaction provides information about the amount and types of social behavior with another animal, including positive (e.g., grooming the other rat) and negative (e.g., biting or a boxing stance) interactions. Social interaction was assessed once during adolescence and once during adulthood. Separate ANCOVAs were conducted during adolescence and adulthood with total horizontal activity (in open field) as a covariate to determine if there were any differences when accounting for overall movement differences. This covariate was used because social interaction occurred in the same type of chamber as

open field and because overall total movement could affect total movement in a social context. The dependent variables were total social interaction, total positive social interactions, and total negative social interaction behaviors.

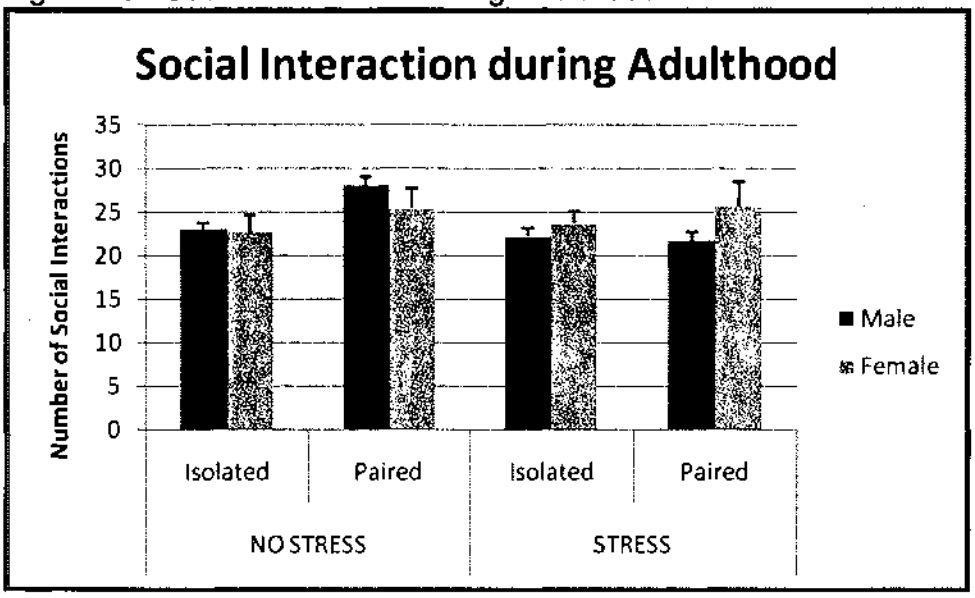
During adolescence, there was an overall trend toward a main effect of stress on total social interaction behaviors ($F [1, 70] = 2.81$, $p = 0.10$) and an internal analysis revealed that only males had a trend toward a stress effect ($F [1, 26] = 3.48$, $p = 0.07$) with rats that were prenatally stressed showing fewer total social interactions (see Figure 24 and Tables 61a-b and 62 - 67). An internal analysis also revealed that females had a stress x housing interaction ($F [1, 33] = 6.79$, $p < 0.05$) showing that females that were prenatally not-stressed and isolated showed the most social interactions compared with all other groups (NSI* > StS > StI* > NSS*). For negative interaction behaviors, there was an overall trend toward a main effect of stress ($F [1, 64] = 3.37$, $p = 0.07$), with only males showing that prenatally stressed rats had fewer negative interaction behaviors ($F [1, 26] = 12.97$, $p < 0.01$). There were no differences among the groups for positive interaction behaviors in adolescence.

Figure 24. Social Interaction during Adolescence



For total social interaction behaviors during adulthood, an internal analysis revealed that prenatally stressed males had fewer total social interactions compared with males that were not prenatally stressed ($F [1, 36] = 11.02$, $p < 0.01$); prenatally isolated offspring had fewer social interactions ($F [1, 36] = 7.05$, $p < 0.05$); and there was a stress x housing interaction for males only ($F [1, 36] = 6.62$, $p < 0.05$) with non-stressed social rats showing the most social interactions (see Figure 25 and Tables 68 - 74). For negative behaviors, there was an overall main effect of prenatal stress ($F [1, 70] = 12.78$, $p < 0.01$) showing that prenatally stressed rats had fewer social interactions and this effect was true for males ($F [1, 36] = 4.16$, $p < 0.05$) and females ($F [1, 33] = 9.05$, $p < 0.05$). For positive behaviors, an internal analysis revealed that males had a stress x housing interaction ($F [1, 36] = 6.57$, $p < 0.05$) with stressed and social offspring showing the least amount of social interactions (NSI* $\geq$ NSS > StI > StS*).

Figure 25. Social Interaction during Adulthood



Summary of Social Interaction. Prenatal stress appears to decrease total social interaction in adolescence and adulthood but only for male offspring. In addition, male offspring that were isolated during the prenatal and weaning periods appear to have fewer social interaction behaviors during adulthood. Prenatal stress also appears to increase negative social interaction for male offspring in adolescence and adulthood and for females only during adulthood.

Summary of Behavioral Data

To summarize the behavioral variables, the major findings are discussed in this section. In addition, two tables are presented. The first two tables (Table D & E), at the end of this section, presents the major findings. The third table (Table 75), in the appendix, summarizes all behavioral findings.

Prenatal stress, prenatal and weaning housing condition, and sex of the offspring all had significant behavioral effects in later adulthood. Specifically **prenatal stress increased negative social interaction for both sexes** in

adulthood and **decreased total social interaction behaviors** and **food consumption for male offspring** only.

Prenatal and early social housing in male offspring led to: (1) **increased food consumption**; (2) increased immobility time in the forced swim test (an index of **greater depression**) compared with offspring that had mothers housed in isolation during these early life periods; and (3) **increased social interactions** in adulthood. Prenatal and weaning housing environment only affected females on elevated plus maze with **females demonstrating less anxiety-like behavior**.

Male offspring (compared with female offspring): (1) **ate more**; (2) had **less horizontal activity** compared with female offspring; (3) spent less time in the open arms of the elevated plus maze (an index of **greater anxiety**); and (4) spent more time immobile in the forced swim test (an index of **greater depression**).

There were several interactions of prenatal stress, prenatal and weaning housing environment, and sex on the behavioral dependent variables. However, none of these interactions were consistent.

Table E. Significant Behavioral Main Effects

Variable	Stress Main Effect			Housing Main Effect			Sex Main Effect
	Overall	♂	♀	Overall	♂	♀	
Food Cons.	-----	NS > St	-----	-----	-----	-----	M > F
OF – horiz	-----	-----	-----	-----	I < S	-----	M < F
OF Ctr Time #4	-----	-----	-----	-----	I < S	I > S	-----
EPM Adult (open arm)	-----	-----	-----	-----	-----	-----	M < F
FST Adol (immobile)	-----	-----	-----	I < S	I < S	-----	-----
FST Adult (immobile)	-----	-----	-----	I < S	I < S	-----	M > F
Social Int. Adol Neg Beh	-----	NS > St	-----	-----	-----	-----	-----
Social Int (SI) Adult Total	-----	NS > St	-----	-----	I < S	-----	
SI Adult Neg Beh	NS < St	NS < St	NS < St	-----	-----	-----	-----

Table F. Significant Behavioral Interactions

Variable	Overall	♂	♀	Interaction Details
Food Cons.	Stress x Housing	-----	-----	NSM > StM > StF > NSF
OF – horiz	Housing x Sex 3 way	Stress x Housing	-----	Male: NSS > StS = StI > NSI
OF Ctr Time #3	3 way int.	Stress x housing	-----	Male: NSI > StS > NSS = StI
OF Ctr Time #3	3 way int.	-----	Stress x housing	Female: NSI > StS = StI > NSS
OF Ctr Time Ratio #3	3 way int.	-----	-----	Male: StI > NSS > NSI > StS
EPM Adol (open arm)	Stress x housing	Stress x housing	-----	Male: NSS > StI > StS > NSI
EPM Adult (open arm)	3 way int.	-----	Stress x housing	Female: StI > NSS > NSI > StS
FST Adol (immobile)	Sex x housing	NS > St	-----	SM > IM > SF > IF
FST Adol (immobile)	Sex x housing	NS > St	-----	SM > IM > SF > IF
SI Adol Total	-----	-----	Stress x housing	Female: NSI > StS > NSS > StI
Social Int. Adult Total	-----	Stress x housing	-----	Male: NSS > StI = StS = NSI
Social Int. Adult Pos.	-----	Stress x housing	-----	Male: NSI > NSS > StI > StS

ASSESSMENT & DISCUSSION

Assessment of Study Hypotheses

There present experiment had eight hypotheses: (1) body weight/food consumption; (2) biochemical measures of stress (i.e., corticosterone); (3) biochemical measures of cardiovascular health (serum glucose, insulin, cholesterol, and C-Reactive Protein); (4) heart structure; (5) locomotor open field activity; (6) indices of anxiety (locomotor center time and elevated plus maze); (7) index of depression (swim test); and (8) social interaction.

Hypothesis 1: Body Weight and Food Consumption

Hypothesis 1a: Stress. Prenatal stress will decrease body weight and food consumption: **partially supported.** There was a significant overall main effect for stress for body weight, such that prenatal stress decreased body weight in both sexes in weaning, but in males only later in life. Prenatal stress decreased food consumption for males only.

Hypothesis 1b: Housing. Prenatal and weaning housing in a social environment will decrease body weight and food consumption compared with housing in an isolated environment: **not supported.** There was no housing main effect for body weight or food consumption.

Hypothesis 1c: Sex Differences. Males will weigh more and eat more than females: **supported.**

Hypothesis 2: Biochemical Measure of Stress

Hypothesis 2a: Stress. Prenatal stress will increase serum corticosterone in adult offspring: **supported**. Prenatal stress led to higher serum corticosterone levels in both sexes compared with offspring who were not prenally stressed.

Hypothesis 2b: Housing. Social housing environment will lower corticosterone levels compared with an isolated housing environment: **not supported**. There was no main effect of housing on serum corticosterone levels in adult offspring.

Hypothesis 2c: Stress and Housing. Social enrichment during gestation and weaning will attenuate increased corticosterone in the offspring: **not supported**. There was no stress x housing interaction for serum corticosterone levels.

Hypothesis 2d: Sex Differences. Female rats will have higher corticosterone levels than male rats: **supported**.

Hypothesis 3: Biochemical Measures of Cardiovascular Health

Hypothesis 3a: Stress. Prenatal stress will increase serum glucose, insulin, cholesterol, and C-Reactive Protein levels in offspring: **not supported**. Prenatal stress decreased serum glucose levels and decreased CRP in adult offspring (in males). There were no overall effects of prenatal stress on cholesterol or insulin.

Hypothesis 3b: Housing. Prenatal and weaning housing environment will decrease serum glucose, insulin, cholesterol, and C-Reactive Protein levels in adult offspring compared with offspring from an isolated housing environment:

partially supported. Social housing did not have an impact on serum glucose and cholesterol, but it did affect CRP and insulin in different directions. Social enrichment decreased levels of CRP (consistent with the hypothesis) and increased levels of insulin (inconsistent with the hypothesis).

Hypothesis 3c: Stress and Housing. Social enrichment will attenuate the increases in serum glucose, insulin, cholesterol, and C-Reactive Protein levels: **not supported**. There were no stress x housing interactions for serum glucose, cholesterol, or insulin levels. There was a stress x housing interaction for CRP, but not in the hypothesized direction. Offspring that were not stressed and isolated had the highest CRP levels compared with all other groups.

Hypothesis 4: Heart Morphology

Hypothesis 4a: Stress. Prenatal stress will change the structure of the offspring's heart; specifically, offspring that were prenatally stressed will have reduced heart lengths and left ventricles, and thicker septal walls: **partially supported**. Prenatal stress decreased heart length and increased lateral wall thickness. The decreased length was true for both sexes, whereas the increased lateral wall thickness was only true for males. There were no effects of prenatal stress on left ventricle or septal wall.

Hypothesis 4b: Housing. Social housing environment will decrease septal wall thickness and increase heart length: **not supported**. A social housing environment decreased heart length for both sexes and increased septal wall thickness and posterior wall thickness for females only.

Hypothesis 4c: Stress and Housing. Prenatal enrichment will attenuate the effects of prenatal stress on heart weight, length, septal wall,, and posterior wall thickness: **partially supported**. There were a few trends toward significant stress x housing interactions on the heart dimensions of right ventricle, septal wall, and anterior wall but the direction of these interaction did not indicate that housing attenuated stress.

Hypothesis 4d: Sex Differences. Male offspring will have longer hearts and thicker left ventricular walls compared with female offspring: **not supported**. There were differences in heart structure by sex, but not on the dimensions hypothesized. Instead, males had heavier hearts, larger right ventricles, and thicker lateral walls compared with female offspring (even when controlling for body weight).

Hypothesis 5: Open Field Locomotor Behavior

Hypothesis 5a: Stress. Prenatal stress will decrease activity levels in the locomotor open field chamber compared with rats not exposed to prenatal stress: **not supported**. There was no effect of prenatal stress on horizontal activity.

Hypothesis 5b: Housing. Social housing will decrease horizontal activity compared with an isolated housing environment: **not supported**. Males had a housing main effect, but it was in the opposite direction as predicted. In other words, males from a social environment had greater horizontal activity.

Hypothesis 6: Anxiety-Like Behavior

Hypothesis 6a: Stress. Prenatal stress will increase anxiety-like behaviors (as assessed by decreased center time in an open field chamber and

decreased time spent in the open arms) compared with offspring not exposed to prenatal stress: **not supported**. There were no main effects for prenatal stress on open field center time or EPM.

Hypothesis 6b: Housing. Social enrichment will decrease anxiety-like behaviors: **partially supported.** For the last open field measure, a social environment resulted in less anxiety-like behavior for females, but more anxiety-like behavior for males.

Hypothesis 6c: Stress and Housing. Social enrichment will attenuate the effects of prenatal stress on anxiety: **not supported**. For center time in open field, there were no consistent interactions. For EPM, females that experienced prenatal stress and isolation had the least amount of anxiety compared with all other groups.

Hypothesis 6d: Sex Differences. Female rats will have more anxiety-like behaviors than male rats: **not supported**. There was a main effect for sex on the second EPM assessment (during adulthood), however, it was in the opposite direction than predicted. Female offspring had less anxiety compared with male offspring.

Hypothesis 7: Depression-Like Behavior

Hypothesis 7a: Stress. Prenatal stress will increase depressive-like behaviors (as assessed by increased immobility in the forced swim test) compared with rats not exposed to prenatal stress: **not supported**. There was no main effect of prenatal stress on forced swim test.

Hypothesis 7b: Housing. A social housing environment will decrease depressive-like behaviors compared with isolated housing: **not supported.** There was a main effect of prenatal and weaning housing environment on FST, but in the opposite direction as predicted. Social housing during gestation and weaning led to more depressive behavior compared with an early isolated environment. This effect was true for both sexes during adolescence, but changed in adulthood to only include male offspring.

Hypothesis 7c: Stress and Housing. Social enrichment will attenuate the effects of prenatal or early life stress on depressive behaviors: **not supported.** Female offspring that were prenatally stressed and housed in isolation showed the least amount of depressive behavior.

Hypothesis 7d: Sex Differences. Females will have greater depressive behaviors than males: **not supported.** There was a main effect of sex, but in the opposite direction as predicted. Males had greater depressive behavior than females.

Hypothesis 8: Social interaction

Hypothesis 8a: Stress. Prenatal stress will decrease overall social interaction and increase negative social interactions: **partially supported.** There was a trend toward a significant effect of prenatal stress decreasing total social interaction in adolescent males and there was a significant effect in adult males. Prenatal stress decreased negative interaction for adolescent males but increased negative interaction for adult males and females.

Hypothesis 8b: Housing. Social Housing will increase positive social interaction behaviors: **not supported**. There was no main effect of prenatal and weaning housing environment on social interaction.

Hypothesis 8c: Stress and Housing. Social environment will attenuate the decrease in social interaction that occurs because of stress: **not supported**. There were no consistent stress x housing interactions.

Hypothesis 8d: Sex Differences. Females will have less total social interaction behaviors than males: **not supported.** There was no main effect of sex.

Discussion

The goal of this doctoral dissertation research was to use a rat model to determine if: (1) prenatal stress increases subsequent risk factors for mental and physical health, and (2) a social intervention (i.e., social enrichment) during the prenatal period can attenuate any later life detrimental health consequences of prenatal stress. The independent variables were stress (or no stress) during pregnancy, isolation or pair housing (i.e., social enrichment) of the dam during pregnancy, and male or female offspring in a 2 x 2 x 2 full factorial experimental design. The offspring were the subjects of interest. The dependent variables were biological (body weight, serum corticosterone, glucose, insulin, cholesterol, C-Reactive Protein, and heart morphology) and behavioral (food consumption, open field locomotor activity, elevated plus maze, forced swim test, and social interaction) variables relevant to cardiovascular diseases.

There were several major findings from this research project: (1) *prenatal* stress has long-term biological and behavioral consequences; (2) a social prenatal and weaning environment also has long-term biological and behavioral effects but in the opposite direction as predicted; (3) males appear to be more vulnerable to the effects of prenatal stress and early social environment compared with females; and (4) a social environment does not appear to attenuate the long-term effects of prenatal stress.

Each of these findings is discussed in detail below. Following this discussion, relevant methodological issues and study limitations are presented. Finally, possible clinical implications are offered.

Finding #1: Prenatal stress has long-term has biological and behavioral consequences

Prenatal stress affected biology and behavior into adulthood. It was predicted that prenatal stress would have long-term effects on biological and behavioral indices of cardiovascular health. With regard to biology, prenatal stress decreased body weight from birth through adulthood (particularly for males); increased serum corticosterone; decreased serum glucose and CRP in adulthood; and changed the structure of the heart. With regard to behavior, prenatal stress decreased food consumption (for males only) and increased negative social interaction during adolescence (for males) and adulthood (for both sexes). Prenatal stress clearly has an effect on biological variables and changes the way rats interact with each other in adolescence and adulthood. It

is not clear whether these effects are positive or negative for long-term health because the results varied in their directions.

The body weight and corticosterone results are consistent with previous animal research reporting that prenatal stress decreases body weight (e.g., Mairesse, Lesage, Breton, Breant, Hahn & Darnaudery, 2007) and increases corticosterone (e.g., Weinstock, et al., 1998; Mairesse et al., 2007; Koehl et al., 1999). The social interaction finding is consistent with previous reports from the human literature that there is a correlation between prenatal stress and externalizing behaviors (e.g., aggression/destruction) in young children (Robinson, Oddy, Li, Kendall, de Klerk, & Silburn et al., 2008). The findings that prenatal stress affects serum CRP, glucose, and change the structure of the heart are new additions to the research literature.

The present research findings indicate that the effects of prenatal stress on physical health and social interaction extend into adulthood. This finding is striking. The fact that a stressor, occurring when an individual is in the womb, can alter one's risk for later disease and alters adult social behaviors highlights the importance of a pregnant mother's experience on their offspring's lives.

Several explanations could account for why prenatal stress may have a long-term impact. First, prenatal stress may have a direct effect on the offspring by affecting biology *in utero*. Exposure of prolonged stress hormones to the developing brain could result in hyperactivity or hyperreactivity of the offspring's stress system (Chrousos, 2008). Weinstock and colleagues (1992) suggested that prenatal stress changes the feedback regulation of corticosterone in

offspring or alters glucocorticoid receptors in the brain. A second explanation is that there could be an indirect biological effect such that the dams' biology during gestational stress is altered, thereby altering the offspring's biology. Smith, Seckl, Evans, Costall, and Smythe (2004) reported that dams stressed during pregnancy showed hypersecretion of ACTH and corticosterone in response to an acute stress challenge. Third, there could be indirect behavioral effects whereby the dams' behavior is altered during weaning, which in turn, affects the offspring. For example, Smith and colleagues (2004) reported that dams stressed during pregnancy showed depressive behavior and altered nursing behavior.

The profound impact of prenatal stress on biology and behavior into adulthood in this study suggests that prenatal stress needs more research and clinical attention. Future research should determine if prenatal stress has a detrimental or a beneficial effect on long-term health. Clinically, it is also important to consider these findings. If a stressor during this period does indeed have such a long-term impact, then stress during this sensitive period of development may need to be carefully monitored.

Finding #2: A social prenatal and weaning environment has long-term biological and behavioral consequences

The second major finding of the present experiment was that a social prenatal and weaning environment also had long-term biological and behavioral consequences. It was predicted that a social environment would decrease body weight, corticosterone, cholesterol, CRP, glucose and insulin and that it would produce changes in the heart. It also was hypothesized that a social

environment would decrease food consumption, anxiety-like behavior, depressive-behavior, and increase social interaction. Almost all of these hypotheses were wrong. The social environment did have long-term effects, but they were largely in the opposite direction as predicted. With regard to biology, the social environment increased insulin (in males) and changed the heart (possibly in a negative direction). It did decrease CRP in both sexes as predicted. With regard to behavior, the social environment decreased overall horizontal behavior (for males) and increased anxiety-like and depressive-like behavior for males. There were a few behavioral findings that were in the predicted direction, but they were sex specific. A social environment decreased anxiety-like behavior for females only and increased total social interaction in adulthood for males only.

It is known that social enrichment in rats during various life periods has beneficial effects. Adolescent rats that were prenatally stressed, yet raised in an enriched environment, increased the amount of positive play behavior and had reduced pro-inflammatory markers (Laviola, Rea, Morley-Fletcher, Di Carlo, Bacosi, & De Simone et al., 2004). Environmental enrichment in rats also has been shown to reverse the effects of reduced maternal care in offspring. Specifically, enrichment in this population improved hippocampal development (Bredy, Zhang, Grant, Diorio, & Meaney, 2004), cognition (Bredy, Humpartzoomian, Cain, & Meaney, 2003), and stress reactivity (Francis et al., 2002). To date, there is little research examining the effects of social enrichment during pregnancy on the offspring's later health. There is one report that

maternal environment enrichment during pregnancy can exert a beneficial influence on maze learning ability in the offspring (Kiyono, Seo, Shibagaki, & Inougye. 1985). However, in the present experiment, the effect of social enrichment during the prenatal period was complicated by the fact that this social enrichment extended into the weaning period. The extension of this enrichment led to a greater social complexity. During the weaning period cross fostering was noted. In other words, once the dams delivered in the social housing condition, one dam would start gathering the pups and nursing. Then, the other dam would often go over to gather pups by pulling them away from the nursing dam to make her own pile. This back and forth care appeared to occur throughout the weaning period and may have had a profound impact on the pups and the dams. In one cage, one of the dams even ceased nursing and no longer produced milk towards the end of the weaning period. Once this occurred, the other dam was responsible for nursing all pups. This cross-fostering situation could have led to competition for food and/or attention among the pups or it may have evoked an instinctual response in the dams to protect their pups. Had this cross-fostering not occurred, it is unclear if social enrichment during pregnancy only would have been beneficial to the offspring.

The social environment also may have appeared to have negative health consequences because a social environment during pregnancy could be different from a social environment during other life-periods for the dams because: (a) pregnancy hormones alter the effects of social interaction; or (b) social interactions in pregnancy and/or weaning were increased to a level not

experienced prior to pregnancy. Pregnant dams have been reported to be more aggressive than non-pregnant female rats and it has been shown that increased levels of progesterone and estradial produced by the ovaries during gestation are responsible for this increased aggression (Albert & Walsh, 1995). There also are reports that matemal aggression is common when a female has a litter. After a litter has been removed, aggression levels subside and eventually terminate (Erskine et al., 1978). In Erskine and colleagues experiment, rats were pair housed with a "stranger rat" immediately upon arrival and once the dams delivered, the two pregnancy dams went from just two rats per cage to 22-38 rats per cage. This large increase in social interaction around pregnancy and deliver anecdotally occurs in humans as well. Women may find themselves interacting more with family and friends after the birth of a baby. If these increased interactions (for rats or humans) are much greater than the pre-pregnancy amount of social interaction, then the increased social contact could be a significant stressor.

A second reason why social support may have been negative could be a male vs. female difference in the offspring. The present findings suggest that social support appeared to be worse for the male rat offsprings' health (i.e., increased insulin, increased depressive and anxiety-like behavior) than for the female offspring. Therefore, it could be that social support early in life is beneficial for females, but harmful for males. If true, then this finding would be consistent with some previous reports that male rats housed in social conditions had higher corticosterone levels than males housed in isolation, whereas female

rats housed in isolation had higher corticosterone concentrations than female rats housed in social conditions (Brown & Grunberg, 1995). In other words, female rats appear to have stress-decreasing effects from social housing conditions, whereas males appear to have stress-increasing effects from social housing conditions.

Finding #3: Males appear to be more vulnerable to the long-term effects of prenatal influences

There were numerous sex differences in the current experiment. Prenatal stress and prenatal and weaning social environment both had profound effects on long-term behavior and biology regardless of sex. These prenatal influences differed by sex and affected males more than females. It was predicted that there would be some sex differences. Specifically for biological variables, males would eat more, have decreased corticosterone and larger hearts compared with females. For behavioral variables, males would eat more, have less anxiety-like and depressive-like behavior, and greater social interaction compared with females. The sex differences hypothesized for the biological variables and food consumption were confirmed (i.e., males weighed more, had lower corticosterone values, and larger hearts than females). However, there were two effects that were completely unexpected: (1) males showed more depressive- and anxiety-like behavior than females; and (2) males were more affected by prenatal stress and social enrichment than were females (i.e., males were more vulnerable or, females were more resilient). For prenatal stress, males were affected by three of the seven variables biological variables and two out of the four behavioral

indices whereas females were affected by one of the seven biological variables and one of the four behavioral indices. For social enrichment, males were affected by 3/7 biological variables, and 4/4 behavioral indices, whereas females were only affected by 2/7 biological and 1/4 behavioral indices (see Tables 75a-b).

Sex differences on behavioral dependent variables in the rodent literature are widely reported. It is common for males to weigh more and eat more and have lower corticosterone levels compared with females (e.g., Faraday, 2000; 2002; Doremus-Fitzwater, Varlinskaya, & Spear, 2009). These sex differences on body weight, food consumption, and corticosterone also have been reported in response to prenatal stress (e.g., Weinstock et al., 1992; Ordyon & Pivina, 2004). Therefore, the findings that males had increased body weight and food consumption and decreased corticosterone compared with females served as a validation check that the prenatal stress was indeed a stressor. The fact that males showed more depressive- and anxiety-like behavior compared with females was inconsistent with human epidemiological data that women have up to three times the rate of anxiety and depression compared with men (WHO, 2009). Although, the findings that female rats showed less anxiety-like behavior (on EPM) (Pallares, Bernasconi, Feleder, & Cutrera, 2007) and less depressive like behavior (in FST) have been reported in the rodent literature (Mueller & Bale, 2008; Alonso et al., 1991).

There are findings from the human literature that prenatal stress is correlated with increased cortisol, hyperactivity or behavior, and lower baseline

cortisol (but higher reactivity in response to an acute stressor) in both sexes (Bruijn, van Bakel, & van Baar). There also is human literature reporting that prenatal stress is correlated with adolescent depression in females only and ADHD symptoms in males only (Van den Bergh, Van Calster, Van Huffel, & Lagae, 2008). The sex differences from the current experiment were consistent with previous rodent literature but not with human correlational reports.

There are several possible reasons for why males may be more vulnerable to prenatal stress: (1) sex hormones in the brain act differently in males versus females during the prenatal period; (2) gonadal steroid levels in the fetus during a critical period may lead to different physiological and/or behavioral responses between males and females; (3) females are inoculated by prenatal stress which increases their stress resistance later in life but males are not inoculated and therefore experience vulnerability later in life. Also, (4) the methodology used to assess the dependent variables could explain the sex differences (see below for more detail).

Sex hormones have a powerful influence on brain development and can be detected in the first week of gestation (Weinstock, 2007). Sex hormones arrange the neuronal circuitry that makes up the neuroendocrine system and is responsible for behavior. They cause differences in the size of specific brain regions, synaptic connections, and neurotransmitter concentrations (Palanza, 2001). During pre- and neonatal development, the brain undergoes rapid growth making the brain especially vulnerable to hormones (including sex and stress hormones) during this time (Weinstock, 2007). Previous research has reported

that mild prenatal stress affects males and females but the direction of these effects has differed. It has been hypothesized that the HPA axis of prenatally stressed male offspring may take longer than control rats to adapt to repeated exposures ofthe same stressor (Weinstock, 2007). In other words, when rats that were prenatally stressed were exposed to acute stressors postnatally, the HPA axis of females habituated to the acute stress response faster than did the HPA axis of the male offspring, suggesting that males were more vulnerable to an overreactive HPA axis. This lack of habituation to reactivation of the HPA axis could lead to an increased vulnerability to anxiety and depression (McEwen, 2000).

A second potential explanation for sex differences could be that gonadal steroid actions in the fetus during critical periods of development in the womb produce difference reactions between males and females. Ward (1984) reported that prenatal maternal restraint stress modifies gonadal steroid levels in the fetus to the point where it actually induces a masculinization of females. Sachser and Kaiser (1996) reported that female guinea pigs that were prenatally stressed showed more male-typical courtship, play, and social orientation behavior and higher testosterone compared to females that were not prenally stressed. Weinstock (2001) reported that prenatally stressed male rats actually showed a demasculinization and feminization of their sexual behavior. If female rats show a masculinization in response to prenatal stress and males show a feminization, then the current findings could be viewed in the context of females having hormones and behaviors that are more aggressive ("fight or flight") and therefore

potentially more self-survival focused rather than "tending and befriending" others during stress as has been suggested by Taylor and colleagues (2000). Prenatally stressed males may not have these same survival behaviors and hormones that normally would have been present. In other words, females may be more resilient as a result of prenatal stress because their hormones and behaviors have become more "fight or flight" with a focus on survival rather than the social affiliation response ("tend and befriend") which may not increase individual survival as well (Taylor et al., 2000).

In addition to alterations in hormones and behaviors from prenatal stress, the female rats may have gained some resiliency because they were inoculated against stress early in life. The idea of inoculation originated in the medical specialty of immunology. In immunology, it is defined as the prevention of a disease by introducing the body to small amounts of a microorganism that cause a virus (Guyton & Hall, 2000). The idea of inoculation was introduced to social psychology in the 1960s. The inoculation effect states that attitudes and beliefs are vulnerable to persuasive attack by opposing arguments. Therefore, exposing individuals to small doses or weak forms of an attacking message can protect them from these arguments (McGuire, 1964). This inoculation effect has been extended to health psychology and researchers have reported that stressful life events "inoculate" an individual against exaggerated physiological responses to future stressors. There is evidence for this effect in animals (Eysenck, 1983) and humans (Musante, Treiber, Kapuku, Moore, Davis & Strong, 2000; Boyce & Chesterman, 1990). If this effect is at work in the present experiment, then the

findings that females had less anxiety-like behavior and depressive-like behavior makes sense as they would have been inoculated against stress while in utero. This effect may not apply to male rats that were prenatally stressed.

Potential Methodological Explanations. Almost all human epidemiological data report that women have up to three times the rate of anxiety and depression compared with men (WHO, 2009). The prior two hypotheses (masculinization and resilience in females) could help explain why the current experiment found the opposite – that males had more depressive and anxiety-like behavior. Alternatively, there could be methodological issues with the current experiment. For example, EPM and FST may not truly index anxiety or depression, respectively, or they may only index one aspect of anxiety or depression. For example, there is some research to suggest that EPM is a better index of panic rather than other types of anxiety (e.g., generalized anxiety) (Bourin, 1997) and that FST is not the best index of depression (Borsini & Meli, 1988). Moreover, these tests may only assess one construct of anxiety or depression. Forced Swim Test assesses learned helplessness, which is only one aspect of depression. Clinical depression (as defined by the DSM-IV TR) lists 15 possible criteria for depression. It could be that males are more sensitive than females to panic and feelings of helplessness, but that females may portray a different constellation of anxiety and depression symptoms. As a separate but related point, the current findings from the experiment could be accurate, but the clinical criteria for anxiety and depression may capture female symptoms more than male symptoms. It is interesting to note that the present anxiety- and

depressive-like findings relied purely on the observation of animal behavior whereas a depression diagnosis in humans mainly relies on self-report with only some behavioral observations. It could be that human males self-report symptoms that are not consistent with DSM-IV TR's adult criteria for depression. For example, there are reports that men who are depressed may have twice the anger episodes compared with women who were depressed (Winkler, Pjrek, & Kasper, 2006) and anger is not a symptom of depression as defined by the DSM-IV TR. If the current DSM-IV TR criteria do reflect female depression symptoms more often than male depression symptoms, then it may be more difficult for clinicians to detect depression among male patients especially when relying heavily on self-report.

An alternative explanation for the sex differences could be that females experience depression and anxiety symptoms more often, but that males may have more profound symptoms and, therefore, the EPM and FST only detected extreme anxiety-like and depressive like behavior. Males commit suicide at about four times rate of women and represent almost 79% of all U.S. suicides (Centers for Disease Control [CDC], 2005). The increased suicide rate among men has always been assumed to be the result of them using more lethal means (CDC, 2005), but it could be that their symptoms of depression are more intense, causing them to turn to suicide.

An additional methodological explanation of the opposite sex finding in this experiment exists. The difference could be the result of studying rats rather than humans on mental health indices.

These sex differences need additional research and clinical attention. If the finding that females that were prenatally stressed and isolated seem to experience less depression and anxiety generalizes to humans, then it could imply that males with a history of prenatal stress may be considered a particularly vulnerable subgroup to anxiety and depression. If true, then health-care practitioners could take preventive measures.

Finding #4: Prenatal social environment does not attenuate detrimental consequences of prenatal stress

It was predicted that social enrichment would attenuate any detrimental effects of prenatal stress and this was not found in the current experiment. There were several statistical stress x housing interactions, but there was no clear, consistent pattern between variables. There have been no experiments to date, human or animal, that have examined prenatal stress and prenatal social enrichment in the same experiment.

There are several possible reasons why social enrichment did not attenuate the long-term effects of prenatal stress in the current experiment: (1) it is not clear that prenatal stress was unequivocally negative and needed attenuation; (2) if the effects of prenatal stress needed to be attenuated, the social enrichment lasting through the weaning period may have confounded the results; or (3) social enrichment does not attenuate stress and other interventions need to be tested.

This experiment used a mild stressor. It is not clear exactly how intense the stressor. The dams did not vocalize or shake during the stressor, yet they

did have an increased corticosterone response compared with non-stressed rats. As a result, the stress appeared to be moderate. The Yerkes-Dodson Law (1908) uses an inverted U-shaped curve to describe the relationship between arousal and responses. Moderate arousal often improves performance, whereas extreme arousal has detrimental effects on performance. DiPietro and colleagues (2006) reported that higher levels of prenatal anxiety, nonspecific stress, and depressive symptoms were associated with more advanced motor and cognitive development in children 2 years of age. DiPietro found that higher levels of anxiety and stress actually enhanced fetal maturation in healthy populations (DiPietro, Novak, Costigan, Atella, & Reusing, 2006). In the present experiment, the mild-moderate prenatal stress may have similarly been beneficial to the offspring. A dose-response curve of various levels of prenatal stress should be investigated to determine the outcome of varying levels of prenatal stress on physical and mental health in the offspring.

A second reason why social enrichment may not have attenuated prenatal stress could be that the prenatal social enrichment extended throughout the weaning period rather than occurring only prenatally. As previously mentioned, cross-fostering was noted during the weaning period and one dam even ceased nursing which left the other dam to have to nurse two litters of pups. This back and forth weaning could have been a stressor for the dams and/or the pups. As a result, future studies should be designed to compare the effect of social enrichment prenatally versus social enrichment during the postnatal period.

A third reason why social enrichment may not have attenuated prenatal stress is that the social enrichment was too mild or that social enrichment does not attenuate stress. The social enrichment in the present experiment was a very simple manipulation; it was only pair housing. Enrichment can include multiple animals/cage or multiple animals per cage plus toys. Despite the various ways that enrichment can be manipulated, it may not actually attenuate prenatal stress. Future research is needed to determine whether social enrichment decreases prenatal stress. In rats that were prenatally stressed, it is noteworthy that enrichment during postnatal adolescence reversed high depressive-like behavior in FST (Yan, Li, Liu, Li, Li, & Yang et al., 2006) and decreased pro-inflammatory cytokines (Laviola, Rea, Morley-Fletcher, Di Carlo, Bacosi, & De Simone et al., 2004). These studies suggest that social enrichment does have some merit, but future research is needed to examine types of and timing of social enrichment.

Alternatively, social enrichment may not be effective at managing prenatal stress during the prenatal period. Other sources of stress management may be needed. Possible methods of stress reduction in future rodent research include altering light/dark schedule to include a longer sleep (i.e., light) cycle as a way of increasing sleep; decreasing external noise, light and handling stimulation as much as possible; using music or exercise; possibly altering diet to increase carbohydrates and therefore increase serotonin release; and possibly administration of drugs that have been shown to be reasonably safe during human pregnancy (e.g., antidepressants such as sertraline [Mayo Clinic, 2007]).

In summary, more research is needed to determine what levels of prenatal stress (if any) are truly detrimental to long-term health effects in offspring. If negative effects do occur, research is needed to determine if social enrichment can attenuate the detrimental effects of prenatal stress or if other techniques are needed.

Limitations

This research offers interesting new findings about the impact of prenatal stress on physical and mental health throughout the lifespan. There are certain limitations in the present research that should be considered. These limitations include: (1) restricted assessments of corticosterone for both the offspring and the dams; (2) a gross assessment of the heart structure and the biological indices of insulin and cholesterol; (3) a prenatal social environment that lasted through the weaning phase; and (4) a stressor that was only a mild to moderate stressor. Each of these limitations is discussed below.

Serum corticosterone provides a valid and widely used biological marker of stress responses. It was assessed post-mortem in this experiment. Repeated assessments of corticosterone from the offspring throughout the experiment would have provided more information about how the effects of prenatal stress may have differed throughout the development. However, methods for conducting repeated assessments of corticosterone (e.g., tail vein puncture) are invasive and require restraint techniques and venipuncture that are likely to be stressful to the animals and thereby confound the results of an experiment assessing stress versus no stress (Hem et al., 1998). There also were no

assessments of the dams' corticosterone levels during this experiment. An assessment of corticosterone from the dams right after birth as well as after weaning would have provided more information about the dams' stress levels. The only way to have conducted such an assessment would have been to temporarily remove the dams from the pups which would likely stress both the dams and the pups and could have even resulted in pup death (Suckow et al., 2003). These assessments may have provided more information about whether the social enrichment (or isolation) actually served as a stressor during times of parturition or weaning. As methods for repeated and non-invasive corticosterone measures become more available, future studies should examine corticosterone in the offspring throughout the life-span and in the dams during gestation, parturition, and weaning. One method currently being investigated uses frequent fecal samples to determine corticosterone levels and could be a promising approach for multiple, non-invasive, nonstressful, corticosterone measurements (Royo et al., 2004; Cavigelli, Guhad, Ceballos, Whetzel, Nevalainen, Lang, et al., 2006).

In the current experiment, the offspring's hearts were assessed by gross measures of length and thickness using digital calipers. An assessment of heart function, in addition to gross structure, would have provided more information about the effects of prenatal stress on the heart and whether these effects were beneficial or detrimental. Future studies should examine heart structure as well as function to gain a deeper understanding of the effects of prenatal stress on heart disease risk. New techniques like perfusion imaging, delayed

enhancement, or tag imaging are emerging as ways to examine coronary ischemic disease and other cardiopathies in rodents (Vallée, Ivancevic, Nguyen, Morel, & Jaconi, 2004). In the current experiment, total levels of insulin and glucose were assessed to index CVD risk. Insulin resistance would be a better indicator of CVD risk (AHA, 2007). However, insulin resistance is measured by conducting a glucose challenge, which is a procedure that could have potentially added a physiological stressor to the offspring. Given that this experiment was a preliminary investigation of prenatal stress, the goals was to minimize any additional stressors, however future research should consider adding assessments of insulin resistance. Similarly, the preliminary nature of this experiment did not allow for a more sophisticated analysis of cholesterol that would examine HDL and LDL cholesterol. Future research should explore how prenatal stress affects both of these kinds of cholesterol as they both affect CVD risk differently (AHA, 2007).

Another limitation of the present experiment relates to the fact that the prenatal environment lasted through the weaning phase of the experiment rather than ending abruptly at birth. Dams remained in the social condition through parturition to avoid introducing additional stress of changing cages just before parturition. Veterinary staff instructed the experimenter that as long as the dams had adequate space to deliver in the same cage (hence the larger cages for the social condition) being housed together would not be an additional stressor. However, because the dams remained in the same cage through the weaning period, it is unclear if the prenatal environment, the weaning environment, or a

combination led to changes in the biology and behavior of the offspring. Presence of another (social enrichment) was meant and hypothesized to be a positive, but presence of another with newborn offspring may have become a stressor. In this experiment, cross-fostering occurred. Specifically, one dam gather the pups and nurse, then the other dam would often go over to pull pups away from the nursing dam to make her own pile. In one cage, one of the dams even ceased nursing and no longer produced milk towards the end of the weaning period. Once this occurred, the other dam was responsible for nursing all pups. Because of this unexpected cross-fostering, there may have been an increased competition for food or the dams' attention in the social enrichment condition. As a result of the social enrichment extending into the weaning period, this experiment did not completely answer the original research question of whether *prenatal* social enrichment could attenuate any long-term detrimental mental and physical health consequences in the offspring. Future studies still need to examine if *prenatal* social enrichment *per se* has beneficial or detrimental effects. Future research should also clarify what types of social conditions make a difference. There are several ways this research question could be examined: (1) create four groups for the dams – prenatal isolation/weaning isolation, prenatal isolation/weaning social, prenatal social/weaning isolation, prenatal social/weaning social; or (2) create a prenatal and weaning social environment that is divided by clear Plexiglas allowing for the "presence of another," but no risk for cross-fostering. In this situation, the rats could still have social enrichment without the potential threat to the pups' safety and survival. This idea

is worth exploring in the future because social enrichment in animals and social support in humans has been found to be beneficial stress reducer.

Future Research

In addition to the ideas mentioned above, there are three other key areas for future research. First, future studies should use an animal model to examine varying levels of prenatal stress (e.g., mild, moderate, and severe) and follow the offspring into young adulthood using the same dependent variables that were used in the preset experiment. These varying stress levels may help answer the question of whether prenatal stress is beneficial, detrimental or both. Second, future animal research should also examine mechanisms for how prenatal stress leads to biological and behavioral changes in offspring. Gestational stress may permanently alter the reactivity of the HPA axis including increases in CRH in the amygdala and decreases in the number of corticosterone receptors in the hippocampus (Weinstock, 1998; Cratty, Ward, Johnson, Azzaro, & Birkle, 1995). Alterations in the HPA axis is worth continuing to explore. Beyond the HPA axis, there may be stress-protective physiological responses worth investigating. Dehydroepiandrosterone (DHEA) has an ability to block glucocorticoid binding in brain receptors (Sapolsky et al., 2000). Neuropeptide Y (NPY) may also play a protective role against stress because it is thought to facilitate the containment of negative consequences following exposure to stress (Heilig, 2004). Another neuropeptide, oxytocin, also may buffer the stress response. It would be worth exploring if prenatal stress decreases DHEA, NPY, or oxytocin; or if administration of these substances can decrease any negative effects of prenatal

stress. Prenatally stressed offspring that were administered oxytocin into the central amygdala had improved social competence (Lee et al., 2007) and humans given intranasal oxytocin after a stressor experienced an anxiolytic effect and decrease in salivary cortisol (Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003). The potential buffering effects of DHEA, NPY, and oxytocin against stress deserve additional research attention, particularly if they can protect against or reverse any detrimental effects of prenatal stress.

Third, future studies should examine human populations using longitudinal, prospective questionnaires and biological assessments to determine how prenatal stress affects children throughout their lifespan. One population for which prenatal stress may be particularly applicable is the military. This population is ideal for a human investigation of prenatal stress for two main reasons: (1) the population, especially during wartime situations experiences stress, and (2) all military and military dependents have medical records that are comprehensive and electronic. A research study in this group could investigate a group of pregnant women whose partners are deployed in a war zone and then follow their children's long-term physical and mental health. This group of children could then be compared with children from mothers whose partners are also military, but were not deployed during the mother's pregnancy. This study would be valuable because of the stress experienced by this population, the convenience of medical records, and because it could be conducted retrospectively or prospectively.

Based on the findings from the present experiment, it is hypothesized that prenatal stress with humans would increase cortisol, decrease body weight, and change the heart regardless of sex. It is also hypothesized that prenatal stress would increase anxiety and depression in males. Such human investigations would determine if the present findings extend to humans, what complicating factors there may be (e.g., parenting style, genetic predisposition), and where to intervene.

Clinical Implications

The present experiment has several important clinical implications. The findings suggest that prenatal stress not only affects offspring, but it affects them *throughout* their life. The impact of psychological stress on disease is well documented (e.g., Selye, 1976), but relatively little research has examined the long-term effects of prenatal stress (e.g., Weinstock, 1997; Barker, 2004).

The current research also suggests that a prenatal and neonatal social environment impact later mental and physical health. The current findings were that an early social enrichment decreases CRP, increases insulin (for males), change heart structure, and increases anxiety and depressive-like behavior (for males).

If these findings are applicable to humans, then these findings could have important implications for prenatal and neonatal care. What is not clear from the present research and the existing body of literature is if the effects of prenatal stress are detrimental, beneficial, or a combination. Janet DiPietro, a leading researcher in the field of human prenatal stress, cautions that "the assumption

that [prenatal] stress is bad, therefore stress will hurt my baby is unjustified" (DiPietro, 2002, p. 3). Moreover, stress is a matter of perception (Selye, 1976), so what is stressful to one person might not be stressful to another. Therefore, at this stage of research, prenatal stress is probably best addressed individually – a dialogue between patient and doctor about current levels of life stress and appropriate stress management techniques if indicated. It may even be beneficial to develop short, paper and pencil, prenatal stress assessments and put behavioral health specialists in obstetricians' offices to assess and help teach stress-management to vulnerable patients.

If prenatal stress has clear detrimental effects, then there would be additional clinical implications. For example, interventions would need to be designed for pregnant women or young infants or children in an attempt to attenuate or reverse the stress. It is possible that these interventions could be quite simple (e.g, deep breathing). If these simple manipulations of mild-moderate stress and pair housing can lead to life-long changes, then it is feasible that other simple actions (e.g, deep breathing, diet changes) during the prenatal period could also have a life-long impact on offspring. Additionally, the earlier the intervention the better as the goal would be to change the child's lifelong health trajectory.

In addition to changes at the individual level, public policy changes for maternal employment and leave also may be indicated if future research finds prenatal stress to be detrimental. In the United States, only 8% of all workers have access to paid family leave (83% have access to unpaid leave) (U.S.

Department of Labor, 2007). Research has shown that maternity leave is associated with better health for mother and child. Leave at 36 weeks gestation prolonged gestation and reduced primary cesarean deliveries among working women (Guendelman, Pearl, Graham, Hubbard, Hosang, & Kharrazi, 2009). A positive association also has been reported between length of maternity leave and mother's mental health, duration of breast feeding, lower neonatal and child mortality (Stehelin, Bertea, & Stutz, 2007).

If prenatal stress is actually beneficial to the physical and mental health of the offspring, then the clinical implications would be different. Stress-assessment and psychoeducation would remain important actions for clinicians to take. Psychoeducation about the potential benefits of stress during pregnancy would be important because women may have preconceived notions that prenatal stress is harmful. For most women, there is enough naturally occurring stress (eustress and distress) during pregnancy that there probably would be no need to increase it. According to the Holmes and Rahe Social Readjustment Rating Scale (1967), pregnancy is listed as the 12th largest stressor in life. There are many reasons why pregnancy is considered stressful. It is accompanied with bodily changes (including extreme hormone changes that can sometimes result in feeling sick or fatigued), changes in family dynamics (i.e., adding a child), and/or potential work changes. For some women there is anxiety about labor, postpartum concerns, anticipation or occurrence of sleep loss, concerns about the unborn child's health, parenting worries, or financial concerns (Affonso & Mayberry, 1990). If prenatal stress is unequivocally beneficial and these

developmentally normal worries do not produce enough stress, then it might be valuable to increase prenatal stress levels. These increases in stress levels could occur through seeking new challenges (work or personal); increasing exercise; taking on new responsibilities at home, work, or in the community; or inducing mild sleep deprivation.

Intentionally increasing a pregnant woman's stress level to keep mothers' stress levels high is a counter-intuitive clinical implication and not something to be entered into lightly. More research is needed to determine if the long-term physical and mental health effects of prenatal stress are beneficial, detrimental, or depend on the variable. The results of the present experiment are intriguing because they suggest that prenatal stress has some positive and some negative long-term effects. Whether the long-term health effects of prenatal stress are beneficial or detrimental may depend on women's or children's individual differences, which health variables are the outcome of research, or other factors. These are future questions that need serious consideration.

CONCLUSIONS

In summary, prenatal stress and social environment during prenatal and weaning periods were both powerful predictors of long-term physical and mental health. There were differences in biological and behavioral outcomes based on sex with males appearing more sensitive to both prenatal stress and early social environment. Prenatal stress resulted in higher corticosterone levels; increased negative social interactions in adulthood and altered heart morphology for both sexes; and lower body weight, C-Reactive Protein, and glucose for males only. A social prenatal and weaning environment resulted in lower C-Reactive Protein and changes in heart morphology for both sexes; greater insulin, horizontal activity, anxiety, and depressive-like behavior; and a greater amount of social interaction in adulthood for males only. Interestingly, females in an early social condition actually showed less anxiety-like behavior on one of the anxiety indices. There were other clear sex differences showing that males may actually have increased vulnerability to the long-term physical and mental health effects of prenatal stress and/or that females may have a particular resilience. More research is needed to determine if the effects of prenatal stress are indeed detrimental and, if so, what interventions are effective to decrease the long-term effects of prenatal stress. If the current research extends to humans, then it is possible that external influences such as stress and a mother's social environment impact the unborn fetus and that increased awareness may need to be given to the prenatal conditions in an effort to improve that fetus' *long-term* mental and physical health.

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APPENDIX

Table 1. List of Abbreviations Used in Tables	
Abbreviation	**Meaning**
PN	Prenatal
Iso	Isolated
BW	Body Weight
FC	Food Consumption
LV	Left Ventricle
RV	Right Ventricle
SI	Social Interaction
SE	Standard Error of the Mean

Table 2. Experiment Timeline			
Exp Day	**Rat Age**	**Cohort A**	**Cohort B**
1	Gestation Day 4	Dams Arrive/ Iso or Pair House	Dams Arrive/ Iso or Pair House
2	Gestation Day 5	Gentle/BW	Gentle/BW
3	Gestation Day 6	Gentle/ Fox urine stress	Gentle/ Fox urine stress
4	Gestation Day 7	Gentle/ Urine + sound 1*	Gentle/ Urine + sound 1
5	Gestation Day 8	Urine + light stress 1	Urine + light stress 1
6	Gestation Day 9	Urine + sound 2 stress	Urine + sound 2 stress
7	Gestation Day 10	Urine + cage shaking	Urine + cage shaking
8	Gestation Day 11	Urine + sound 3 stress	Urine + sound 3 stress
9	Gestation Day 12	Urine only stress	Urine only stress
10	Gestation Day 13	Urine + cage shaking	Urine + cage shaking
11	Gestation Day 14	Urine + light stress 2	Urine + light stress 2
12	Gestation Day 15	Urine only stress	Urine only stress
13	Gestation Day 16	Urine + sound 4 stress	Urine + sound 4 stress
14	Gestation Day 17	Urine + sound 3 stress	Urine + sound 3 stress
15	Gestation Day 18	Urine + cage shaking	Urine + cage shaking
16	Gestation Day 19	Urine	Urine
19	Gestation Day 22/ Postnatal Day (PN) 0	Parturition	Parturition
40	PN22	Iso house/BW/FC/Handling	Iso house/BW/FC/Handling
41	PN23	Handling/ Open Field Acclimation	Handling
42	PN24	Open Field	Open Field Acclimation
43	PN25		Open Field
46	PN28	BW/FC/Elevated Plus Maze	
47	PN29		BW/FC/Elevated Plus Maze
52	PN34	Social Interaction	
53	PN35		Social Interaction
66	PN48	Open Field	
67	PN49		Open Field
94	PN76	Swim Test	
95	PN77	Swim Test	
96	PN78		Swim Test
97	PN79		Swim Test
101	PN83	Open Field	
102	PN84		Open Field
142	PN124	Social Interaction	
143	PN125		Social Interaction
147	PN128	Open Field	
152	PN129		Open Field
153	PN134	Elevated Plus Maze	
154	PN135		Elevated Plus Maze
155	PN136	Swim Test	
156	PN137	Swim Test	
157	PN138		Swim Test
158	PN139		Swim Test
169	PN150	Sacrifice	Sacrifice

*See Table B in text (page 46) for a description of noise, light and cage shaking descriptions

Table 3. List of Abbreviations for Rat Conditions	
Abbreviation	**Meaning**
NSIM	No-Stress/Isolated Males
NSIF	No-Stress/Isolated Females
StIM	Stress/Isolated Males
StIF	Stress/Isolated Females
NSSM	No-Stress/Social Males
NSSF	No-Stress/Social Females
StSM	Stress/Social Males
StSF	Stress/Social Females

Table 4. Descriptive Values for Dams'
Gestation Weight

	Mean	Std. Error
No Stress/Isolated	202.45	7.06
No Stress/Social	172.95	12.96
Stress/Isolated	198.67	1.25
Stress/Social	210.72	6.53

Table 5. ANOVA for Dams' Body Weight at Start of Experiment
Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Stresscond	261.00	1	261.00	1.42	0.26	0.12	0.19
Housecond	990.57	1	990.57	5.39	0.04	0.35	0.55
Stresscond x Housecond	1479.77	1	1479.77	8.05	0.02	0.45	0.73
Error	1837.63	10	183.76				
Total	550029.68	14					

Table 6. Descriptive Values for Body Weight (g)

Phase	Weaning		Month 1		Month 2		Month 3		Month 4		Final Weight	
Group	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
NSIM	53.7	2.3	121.5	4.7	359.8	14.5	443.0	18.7	483.5	20.4	504.6	22.0
NSIF	51.5	1.9	98.8	2.6	227.5	5.9	262.3	6.7	275.3	7.4	279.5	7.2
StIM	44.7	1.6	103.0	1.7	334.5	7.3	306.3	27.2	448.7	7.7	466.5	8.2
StIF	44.9	1.4	89.2	1.7	215.0	4.3	246.4	5.0	259.8	5.8	268.1	6.3
NSSM	50.1	1.9	112.3	3.0	343.7	7.0	423.8	10.1	462.5	11.4	481.4	11.9
NSSF	48.6	2.1	93.2	3.5	216.7	7.4	270.8	18.7	265.4	8.9	269.2	9.9
StSM	44.7	1.7	106.2	3.2	342.6	10.6	424.2	13.6	464.9	16.2	486.2	17.6
StSF	43.9	2.2	90.5	3.1	217.2	7.1	252.0	8.6	268.6	9.5	277.3	10.0

Table 7. ANOVA for Body Weight on Weaning Day

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Sex	21.65	1	21.65	0.60	0.44	0.01	0.12
Stress	804.00	1	804.00	22.39	0.00	0.24	1.00
Housing	69.48	1	69.48	1.94	0.17	0.03	0.28
Sex x Stress	11.04	1	11.04	0.31	0.58	0.00	0.09
Sex x Housing	0.15	1	0.15	0.00	0.95	0.00	0.05
Stress x Housing	38.19	1	38.19	1.06	0.31	0.02	0.17
Sex x Stress x Housing	3.84	1	3.84	0.11	0.75	0.00	0.06
Error	2549.29	71	35.91				

Table 8a. Repeated-Measures ANCOVA for BW

Tests of Within-Subject Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	3289920	3	1096640	2383.3	.000	.971	1.0
Time x Sex	348750	3	116250	252.6	.000	.781	1.0
Time x Stress	3827	3	1275	2.77	.042	.038	.664
Time x Housing	3041	3	1013	2.2	.089	.030	.554
Time x Sex x Stress	386	3	128	.280	.840	.004	.103
Time x Sex x Housing	358	3	119	.260	.854	.004	.099
Time x Stress x Housing	1641	3	547	1.19	.315	.016	.317
Time x Sex x tress x Housing	3584	3	1194	2.59	.053	.035	.632
Error	98007	213	460				

Table 8b. Repeated-Measures ANCOVA for BW

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Sex	1218327	1	1218327	383.8	.000	.844	1
Stress	15369	1	15369	4.8	.031	.064	.58
Housing	55	1	55	.017	.895	.000	.052
Sex x Stress	2043	1	2043	.644	.425	.009	.124
Sex x Housing	51	1	51	.016	.899	.000	.052
Stress x Housing	9872	1	9872	3.1	.082	.042	.413
Sex x Stress x Housing	3605	1	3605	1.1	.290	.016	.183
Error	225377	71	3174				

Table 9. Repeated-Measures ANOVA for BW – Males Only

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Stress	14864.43	1	14864.43	3.15	0.08	0.08	0.41
Housing	111.26	1	111.26	0.02	0.88	0.00	0.05
Stress x Housing	13197.13	1	13197.13	2.80	0.10	0.07	0.37

Table 10. ANCOVA for Mean Body Weight at End of Experiment

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Weaning	46059.54	1	46059.54	43.69	0.00	0.38	1.00
Sex	826883.99	1	826883.99	784.29	0.00	0.92	1.00
Stress	4884.14	1	4884.14	4.63	0.04	0.06	0.57
Housing	903.05	1	903.05	0.86	0.36	0.01	0.15
Sex x Stress	361.42	1	361.42	0.34	0.56	0.01	0.09
Sex x Housing	19.27	1	19.27	0.02	0.89	0.00	0.05
Stress x Housing	1808.01	1	1808.01	1.72	0.20	0.02	0.25
Sex x Stress x Housing	307.69	1	307.69	0.29	0.59	0.00	0.08
Error	73801.85	70	1054.31				

Table 11. Descriptive Values for Serum Corticosterone (ng/ml)

Group	Mean	Std. Error
NSIM	250.0	36.68
NSIF	558.6	64.23
StIM	260.8	30.61
StIF	671.5	95.79
NSSM	212.7	35.89
NSSF	496.6	77.57
StSM	315.2	65.27
StSF	594.6	54.23

Table 12. ANOVA for Serum Corticosterone

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Sex	1964925.13	1	1964925.13	52.00	0.00	0.43	1.00
Stress	125542.97	1	125542.97	3.32	0.07	0.05	0.44
Housing	17757.43	1	17757.43	0.47	0.50	0.01	0.10
Sex x Stress	11348.94	1	11348.94	0.30	0.59	0.00	0.08
Sex x Housing	29069.67	1	29069.67	0.77	0.38	0.01	0.14
Stress x Housing	7057.23	1	7057.23	0.19	0.67	0.00	0.07
Sex x Stress x Housing	13604.03	1	13604.03	0.36	0.55	0.01	0.09
Error	2645182.14	70	37788.32				

Table 13. Descriptive Values for Serum Cholesterol (ng/ml)

Group	Mean	Std. Error
NSIM	81.2	7.84
NSIF	75.1	4.87
StIM	79.2	6.61
StIF	71.1	5.09
NSSM	85.5	5.63
NSSF	76	4.30
StSM	91.1	9.20
StSF	81.9	8.06

Table 14. ANOVA for Serum Cholesterol

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Sex	1339.74	1	1339.74	2.99	0.09	0.04	0.40
Stress	36.86	1	36.86	0.08	0.78	0.00	0.06
Housing	960.11	1	960.11	2.14	0.15	0.03	0.30
Sex x Stress	3.61	1	3.61	0.01	0.93	0.00	0.05
Sex x Housing	25.14	1	25.14	0.06	0.81	0.00	0.06
Stress x Housing	377.06	1	377.06	0.84	0.36	0.01	0.15
Sex x Stress x Housing	6.50	1	6.50	0.01	0.91	0.00	0.05
Error	32315.29	72	448.82				

Table 15. Descriptive Values for CRP (ng/ml)

Group	Mean	Std. Error
NSIM	41.8	1.30
NSIF	39.5	2.06
StIM	33.4	2.31
StIF	33.1	1.83
NSSM	31.9	0.76
NSSF	31.3	2.06
StSM	29.8	1.77
StSF	30.6	2.06

Table 16. ANOVA for CRP

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Sex	7.37	1	7.37	0.22	0.64	0.00	0.08
Stress	376.75	1	376.75	11.21	0.00	0.14	0.91
Housing	713.42	1	713.42	21.23	0.00	0.23	1.00
Sex x Stress	13.96	1	13.96	0.42	0.52	0.01	0.10
Sex x Housing	9.60	1	9.60	0.29	0.60	0.00	0.08
Stress x Housing	176.08	1	176.08	5.24	0.03	0.07	0.62
Sex x Stress x Housing	0.30	1	0.30	0.01	0.93	0.00	0.05
Error	2385.62	71	33.60				

Table 17. ANOVA for CRP – Males Only

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Stress	204.49	1	204.49	8.45	0.01	0.19	0.81
Housing	530.85	1	530.85	21.94	0.00	0.37	1.00
Stress x Housing	67.43	1	67.43	2.79	0.10	0.07	0.37
Error	895.25	37	24.20				
Total	50109.21	41					

Table 18. ANOVA for Females – CRP

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Stress	121.28	1	121.28	2.96	0.10	0.08	0.39
Housing	269.37	1	269.37	6.58	0.02	0.17	0.70
Stress x Housing	68.92	1	68.92	1.68	0.20	0.05	0.24
Error	1351.43	33	40.95			.	
Total	44201.58	37					

Table 19. Descriptive Values for Serum glucose (ng/ml)

Group	Mean	Std. Error
NSIM	196.2	6.70
NSIF	165.4	7.42
StIM	164.5	8.66
StIF	151	6.96
NSSM	151.8	2.94
NSSF	181.9	8.43
StSM	176.3	11.81
StSF	145.9	7.65

Table 20. ANOVA for Serum Glucose

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Sex	13580.78	1	13580.78	17.46	0.00	0.20	0.99
Stress	4115.14	1	4115.14	5.29	0.02	0.07	0.62
Housing	559.95	1	559.95	0.72	0.40	0.01	0.13
Sex x Stress	358.58	1	358.58	0.46	0.50	0.01	0.10
Sex x Housing	323.67	1	323.67	0.42	0.52	0.01	0.10
Stress x Housing	1474.61	1	1474.61	1.90	0.17	0.03	0.27
Sex x Stress x Housing	382.12	1	382.12	0.49	0.49	0.01	0.11
Error	55996.13	72	777.72				

Table 21. ANOVA for Serum Glucose – Males Only

Tests of Between Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Stress	3442.62	1	3442.62	3.27	0.08	0.08	0.42
Housing	23.58	1	23.58	0.02	0.88	0.00	0.05
Stress x Housing	1654.99	1	1654.99	1.57	0.22	0.04	0.23
Error	38944.75	37	1052.56				
Total	1372559.00	41					

Table 22. Descriptive Values for Serum Insulin (ng/ml)

Group	Mean	Std. Error
NSIM	44.7	2.28
NSIF	32.5	3.06
StIM	44.7	3.32
StIF	27.7	2.34
NSSM	47.9	1.30
NSSF	44.5	4.16
StSM	34.5	2.89
StSF	47.4	3.46

Table 23. ANOVA for Serum Insulin

Tests of Between-Subjects Effects

Source	Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Sex	2400.07	1	2400.07	22.11	0.00	0.25	1.00
Stress	276.20	1	276.20	2.54	0.12	0.04	0.35
Housing	705.45	1	705.45	6.50	0.01	0.09	0.71
Sex x Stress	240.87	1	240.87	2.22	0.14	0.03	0.31
Sex x Housing	195.74	1	195.74	1.80	0.18	0.03	0.26
Stress x Housing	36.12	1	36.12	0.33	0.57	0.01	0.09
Sex x Stress x Housing	25.69	1	25.69	0.24	0.63	0.00	0.08
Error	7381.23	68	108.55				

Table 24. ANOVA for Serum Insulin – Females Only

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Stress	464.90	1	464.90	3.63	0.07	0.11	0.45
Housing	740.11	1	740.11	5.77	0.02	0.16	0.64
Stress x Housing	55.25	1	55.25	0.43	0.52	0.01	0.10
Error	3846.54	30	128.22				
Total	47645.09	34					

Table 25a. Descriptive Values for Heart Morphology (mm)

	Length		Weight		Width		LV		RV	
Group	Mean	Std	Mean	Std	Mean	Std	Mean	Std	Mean	Std
NSIM	18.1	0.3	2.2	0.6	13.3	0.4	4.3	0.3	2.2	0.2
NSIF	15.5	0.3	1.0	0.2	11.1	0.3	3.5	0.2	1.6	0.2
StIM	16.8	0.4	1.5	0.1	12.5	0.4	4.1	0.3	2.0	0.2
StIF	15.0	0.3	1.5	0.4	11.2	0.3	3.6	0.3	1.8	0.2
NSSM	17.0	0.4	1.6	0.1	13.5	0.4	4.7	0.2	2.4	0.2
NSSF	14.8	0.3	1.1	0.0	11.4	0.2	3.6	0.2	1.7	0.2
StSM	16.5	0.2	1.6	0.1	13.4	0.3	4.7	0.3	1.7	0.3
StSF	14.2	0.3	1.0	0.0	11.5	0.2	3.8	0.1	1.6	0.2

Table 25b. Descriptive Values for Heart Morphology (mm)

	Septal		Lateral		Posterior		Anterior	
Group	Mean	Std	Mean	Std	Mean	Std	Mean	Std
NSIM	2.8	0.1	3.0	0.1	3.5	0.2	3.5	0.2
NSIF	2.7	0.1	2.5	0.1	3.0	0.2	3.2	0.1
StIM	2.9	0.2	3.2	0.2	3.4	0.2	3.4	0.2
StIF	2.4	0.1	2.8	0.2	2.8	0.2	3.0	0.2
NSSM	2.8	0.1	2.9	0.1	3.3	0.1	3.1	0.2
NSSF	2.5	0.1	2.7	0.1	3.2	0.2	3.0	0.2
StSM	2.8	0.1	3.3	0.1	3.7	0.2	3.4	0.2
StSF	3.0	0.1	2.8	0.2	3.2	0.2	3.3	0.2

Table 26a. Multivariate Test for Combined Heart Variables

Effect	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared	Observed Power
Final BW	0.49	7.18	9	62	0.00	0.51	1.00
Sex	0.78	1.90	9	62	0.07	0.22	0.78
Stress	0.75	2.36	9	62	0.02	0.26	0.88
Housing	0.62	4.26	9	62	0.00	0.38	0.99
Sex x Stress	0.93	.53	9	62	0.85	0.07	0.24
Sex x Housing	0.93	.50	9	62	0.87	0.07	0.22
Stress x Housing	0.85	1.23	9	62	0.30	0.15	0.55
Sex x Stress x Housing	0.81	1.67	9	62	0.12	0.20	0.71

Table 26b. Heart Morphology
Tests of Between-Subjects Effects

Source	Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Sex	Length	0.04	1	0.04	0.05	0.82	0.00	0.06
	Weight	2.68	1	2.68	3.66	0.06	0.05	0.47
	Width	1.27	1	1.27	1.61	0.21	0.02	0.24
	LV	0.24	1	0.24	0.38	0.54	0.01	0.09
	RV	1.09	1	1.09	3.38	0.07	0.05	0.44
	Septal	0.10	1	0.10	0.78	0.38	0.01	0.14
	Lateral	0.80	1	0.80	5.28	0.03	0.07	0.62
	Posterior	0.00	1	0.00	0.02	0.88	0.00	0.05
	Anterior	0.09	1	0.09	0.53	0.47	0.01	0.11
Stress	Length	7.25	1	7.25	8.94	0.00	0.11	0.84
	Weight	0.17	1	0.17	0.23	0.63	0.00	0.08
	Width	0.11	1	0.11	0.13	0.72	0.00	0.07
	LV	0.06	1	0.06	0.10	0.76	0.00	0.06
	RV	0.48	1	0.48	1.48	0.23	0.02	0.22
	Septal	0.12	1	0.12	0.90	0.35	0.01	0.16
	Lateral	1.13	1	1.13	7.40	0.01	0.10	0.77
	Posterior	0.03	1	0.03	0.17	0.68	0.00	0.07
	Anterior	0.21	1	0.21	1.28	0.26	0.02	0.20
Housing	Length	10.71	1	10.71	13.20	0.00	0.16	0.95
	Weight	1.16	1	1.16	1.59	0.21	0.02	0.24
	Width	3.38	1	3.38	4.29	0.04	0.06	0.53
	LV	2.24	1	2.24	3.59	0.06	0.05	0.46
	RV	0.01	1	0.01	0.04	0.84	0.00	0.06
	Septal	0.26	1	0.26	2.06	0.16	0.03	0.29
	Lateral	0.00	1	0.00	0.03	0.87	0.00	0.05
	Posterior	0.44	1	0.44	2.40	0.13	0.03	0.33
	Anterior	0.02	1	0.02	0.10	0.75	0.00	0.06
Sex x Stress	Length	0.15	1	0.15	0.19	0.67	0.00	0.07
	Weight	1.77	1	1.77	2.42	0.13	0.03	0.34
	Width	0.52	1	0.52	0.66	0.42	0.01	0.13
	LV	0.03	1	0.03	0.05	0.82	0.00	0.06
	RV	0.48	1	0.48	1.49	0.23	0.02	0.23
	Septal	0.00	1	0.00	0.01	0.94	0.00	0.05
	Lateral	0.04	1	0.04	0.26	0.61	0.00	0.08
	Posterior	0.24	1	0.24	1.29	0.26	0.02	0.20
	Anterior	0.04	1	0.04	0.24	0.62	0.00	0.08

Table 26b. Continued - Heart Morphology
Tests of Between-Subjects Effects

Source	Dependent Variable	Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Sex x Housing	Length	0.03	1	0.03	0.04	0.85	0.00	0.05
	Weight	0.01	1	0.01	0.01	0.92	0.00	0.05
	Width	0.37	1	0.37	0.46	0.50	0.01	0.10
	LV	0.71	1	0.71	1.14	0.29	0.02	0.18
	RV	0.00	1	0.00	0.00	0.98	0.00	0.05
	Septal	0.37	1	0.37	2.89	0.09	0.04	0.39
	Lateral	0.08	1	0.08	0.50	0.48	0.01	0.11
	Posterior	0.36	1	0.36	1.96	0.17	0.03	0.28
	Anterior	0.45	1	0.45	2.76	0.10	0.04	0.38
Stress x Housing	Length	0.01	1	0.01	0.01	0.93	0.00	0.05
	Weight	0.26	1	0.26	0.35	0.55	0.01	0.09
	Width	0.00	1	0.00	0.00	0.96	0.00	0.05
	LV	0.01	1	0.01	0.01	0.93	0.00	0.05
	RV	1.21	1	1.21	3.74	0.06	0.05	0.48
	Septal	0.50	1	0.50	3.94	0.05	0.05	0.50
	Lateral	0.01	1	0.01	0.06	0.81	0.00	0.06
	Posterior	0.38	1	0.38	2.06	0.16	0.03	0.29
	Anterior	0.68	1	0.68	4.15	0.05	0.06	0.52
Sex x Stress x Housing	Length	0.39	1	0.39	0.48	0.49	0.01	0.11
	Weight	2.11	1	2.11	2.89	0.09	0.04	0.39
	Width	0.13	1	0.13	0.17	0.68	0.00	0.07
	LV	0.00	1	0.00	0.00	0.96	0.00	0.05
	RV	0.11	1	0.11	0.35	0.56	0.01	0.09
	Septal	0.91	1	0.91	7.10	0.01	0.09	0.75
	Lateral	0.18	1	0.18	1.16	0.29	0.02	0.19
	Posterior	0.08	1	0.08	0.44	0.51	0.01	0.10
	Anterior	0.06	1	0.06	0.37	0.55	0.01	0.09
Error	Length	56.79	70	0.81				
	Weight	51.13	70	0.73				
	Width	55.15	70	0.79				
	LV	43.75	70	0.63				
	RV	22.58	70	0.32				
	Septal	8.95	70	0.13				
	Lateral	10.65	70	0.15				
	Posterior	12.89	70	0.18				
	Anterior	11.44	70	0.16				

Table 27. Descriptive Values for Food Consumption (g)

Phase	Month 1		Month 2		Month 3		Month 4	
Group	Mean	Std	Mean	Std	Mean	Std	Mean	Std
NSIM	115.5	6.9	190.4	5.4	197.2	7.0	175.4	7.0
NSIF	92.6	4.0	137.6	6.0	134.1	2.3	118.2	3.7
StIM	92.7	6.2	172.1	4.9	178.1	4.7	164.9	3.7
StIF	86.9	5.7	136.1	3.4	136.9	3.7	131.9	6.9
NSSM	121.6	2.7	185.3	6.4	188.7	4.2	174.2	5.0
NSSF	90.9	2.0	127.4	4.9	132.1	5.4	122.9	3.7
StSM	114.1	3.1	181.4	3.6	187.3	3.7	169.3	4.2
StSF	102.0	5.7	124.6	7.2	137.5	7.6	123.1	7.3

Table 28a. Repeated-Measures ANOVA for Food Consumption

Tests of Within-Subject Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	143478.88	3	47826.29	303.33	0.00	0.84	1.00
Time x Sex	12197.54	3	4065.85	25.79	0.00	0.31	1.00
Time x Stress	303.45	3	101.15	0.64	0.59	0.01	0.18
Time x Housing	1395.26	3	465.09	2.95	0.03	0.05	0.69
Time x Sex x Stress	48.33	3	16.11	0.10	0.96	0.00	0.07
Time x Sex x Housing	112.58	3	37.53	0.24	0.87	0.00	0.09
Time x Stress x Housing	850.98	3	283.66	1.80	0.15	0.03	0.46
Time x Sex x Stress x Housing	277.74	3	92.58	0.59	0.62	0.01	0.17
Error (time)	27434.74	174	157.67				

Table 28b. Repeated-Measures ANOVA for Food Consumption

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	5105908.08	1	5105908.08	8275.80	0.00	0.99	1.00
Sex	108147.36	1	108147.36	175.29	0.00	0.75	1.00
Stress	782.24	1	782.24	1.27	0.27	0.02	0.20
Housing	233.80	1	233.80	0.38	0.54	0.01	0.09
Sex x Stress	3701.01	1	3701.01	6.00	0.02	0.09	0.67
Sex x Housing	353.31	1	353.31	0.57	0.45	0.01	0.12
Stress x Housing	1086.49	1	1086.49	1.76	0.19	0.03	0.26
Sex x Stress x Housing	344.86	1	344.86	0.56	0.46	0.01	0.11
Error	35784.16	58	616.97				

Table 29. Repeated –Measures ANOVA for Food Consumption – Males Only

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Stress	4574.66	1	4574.66	6.95	0.01	0.17	0.73
Housing	674.01	1	674.01	1.02	0.32	0.03	0.17
Stress x Housing	1540.46	1	1540.46	2.34	0.14	0.06	0.32
Error	22369.62	34	657.93				

Table 30. Descriptive Values for Open Field Horizontal Activity
(# of Beam Breaks)

Phase	Month 1		Month 2		Month 3		Month 4	
Group	Mean	Std	Mean	Std	Mean	Std	Mean	Std
NSIM	6078	746.0	17665	1385.7	20572	1423.0	17102	1290.8
NSIF	7739	680.2	24399	1638.1	25513	1416.4	26446	1469.8
StIM	8291	845.0	19204	1444.5	22041	1598.2	20736	1472.0
StIF	7889	654.3	24235	2662.3	25214	1789.2	23020	1253.2
NSSM	7333	653.3	23015	1455.9	25514	1603.3	23923	1743.0
NSSF	8132	828.8	21919	2557.3	22572	1826.8	19914	1136.8
StSM	7819	801.3	21561	2438.1	22222	1874.9	19702	1578.9
StSF	7716	897.5	24563	1173.8	28613	1813.6	24032	1221.0

Table 31a. Repeated Measure ANOVA for Open Field Activity
Tests of Within-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Time	1.35E+10	3	4.49E+09	277.64	0.00	0.80	1.00
Time x Stress	9176167.96	3	3058723	0.19	0.90	0.00	0.09
Time x Housing	3.00E+07	3	1.00E+07	0.62	0.60	0.01	0.18
Time x Sex	1.03E+08	3	3.43E+07	2.12	0.10	0.03	0.54
Time x Stress x Housing	9169474.572	3	3056492	0.19	0.90	0.00	0.09
Time x Stress x Sex	6.87E+07	3	2.29E+07	1.42	0.24	0.02	0.37
Time x Housing x Sex	8.91E+07	3	2.97E+07	1.84	0.14	0.03	0.47
Time x Stress x Housing x Sex	1.41E+08	3	4.70E+07	2.91	0.04	0.04	0.69
Error (time)	3.44E+09	213	1.62E+07				

Table 31b. Repeated-Measures ANOVA for Open Field Activity

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	1.12E+11	1	1.12E+11	2511.40	0.00	0.97	1.00
Stress	2.48E+07	1	2.48E+07	0.56	0.46	0.01	0.11
Housing	4.69E+07	1	4.69E+07	1.06	0.31	0.02	0.17
Sex	4.67E+08	1	4.67E+08	10.52	0.00	0.13	0.89
Stress x Housing	448277.7	1	448277.7	0.01	0.92	0.00	0.05
Stress x Sex	2.09E+07	1	2.09E+07	0.47	0.50	0.01	0.10
Housing x Sex	2.13E+08	1	2.13E+08	4.79	0.03	0.06	0.58
Stress x Housing x Sex	3.42E+08	1	3.42E+08	7.69	0.01	0.10	0.78
Error	3.15E+09	71	4.44E+07				

Table 32. Repeated-Measures ANOVA for Open Field Activity – Males Only

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Stress	88627.47	1	88627.47	0.00	0.96	0	0.05
Housing	2.39E+08	1	2.39E+08	5.52	0.02	0.13	0.63
Stress x Housing	1.91E+08	1	1.91E+08	4.41	0.04	0.11	0.53
Error	1.60E+09	37	4.32E+07				

Table 33. Repeated-Measures ANOVA for Open Field Activity – Females Only

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	6.10E+10	1	6.10E+10	1332.14	0.00	0.98	1.00
Stress	4.40E+07	1	4.40E+07	0.96	0.33	0.03	0.16
Housing	2.88E+07	1	2.88E+07	0.63	0.43	0.02	0.12
Stress x Housing	1.53E+08	1	1.53E+08	3.34	0.08	0.09	0.43
Error	1.56E+09	34	4.58E+07				

Table 34. Descriptive Values for Center Time in Open Field (sec)

Phase	Open Field 1		Open Field 2		Open Field 3		Open Field 4	
Group	Mean	Std	Mean	Std	Mean	Std	Mean	Std
NSIM	99.0	21.0	790.2	76.1	1495.9	131.0	5250.8	702.0
NSIF	109.4	15.0	848.9	52.3	1251.0	96.4	9607.0	1143.6
StIM	135.2	16.6	718.3	120.1	1023.2	96.7	6165.0	542.6
StIF	135.4	21.0	713.8	51.9	1187.3	137.4	6769.2	302.4
NSSM	128.2	26.1	795.8	81.5	1095.1	79.1	7591.7	693.9
NSSF	148.7	32.9	745.0	70.2	1244.7	97.0	6510.3	716.7
StSM	153.7	22.4	963.2	182.2	1399.8	212.7	6608.4	661.7
StSF	113.5	15.4	777.7	62.2	1121.7	120.5	6773.4	454.2

Table 35. ANOVA for Center Time in the Open Field Chamber – Measurement #1

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Stress	3327.74	1	3327.74	0.67	0.42	0.01	0.13
Housing	5197.57	1	5197.57	1.05	0.31	0.02	0.17
Sex	104.52	1	104.52	0.02	0.89	0.00	0.05
Stress x Housing	6336.44	1	6336.44	1.28	0.26	0.02	0.20
Stress x Sex	6092.04	1	6092.04	1.23	0.27	0.02	0.19
Housing x Sex	1126.08	1	1126.08	0.23	0.64	0.00	0.08
Stress x Housing x Sex	3087.19	1	3087.19	0.62	0.43	0.01	0.12
Error	352128.65	71	4959.56				

Table 36. ANOVA for Center Time in the Open Field Chamber – Measurement #2

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Stress	56.29	1	56.29	0.00	0.98	0.00	0.05
Housing	54149.76	1	54149.76	0.54	0.47	0.01	0.11
Sex	40519.55	1	40519.55	0.40	0.53	0.01	0.10
Stress x Housing	202315.18	1	202315.18	2.02	0.16	0.03	0.29
Stress x Sex	47793.05	1	47793.05	0.48	0.49	0.01	0.11
Housing x Sex	102955.17	1	102955.17	1.03	0.32	0.01	0.17
Stress x Housing x Sex	6231.41	1	6231.41	0.06	0.80	0.00	0.06
Error	7128607.23	71	100402.92				

Table 37. ANOVA for Center Time in the Open Field Chamber – Measurement #3

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Stress	153451.41	1	153451.41	0.89	0.35	0.01	0.15
Housing	11256.73	1	11256.73	0.07	0.80	0.00	0.06
Sex	53479.06	1	53479.06	0.31	0.58	0.00	0.09
Stress x Housing	629143.73	1	629143.73	3.65	0.06	0.05	0.47
Stress x Sex	423.99	1	423.99	0.00	0.96	0.00	0.05
Housing x Sex	2788.51	1	2788.51	0.02	0.90	0.00	0.05
Stress x Housing x Sex	854322.89	1	854322.89	4.96	0.03	0.07	0.59
Error	1.22E+07	71	172230.16				

Table 38. ANOVA for Center Time in the Open Field Chamber – Measurement #4 – Males Only

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Stress	71539.13	1	71539.13	0.34	0.56	0.01	0.09
Housing	1474.99	1	1474.99	0.01	0.93	0.00	0.05
Stress x Housing	1531997.00	1	1531997.00	7.24	0.01	0.16	0.75
Error	7828949.88	37	211593.24				
Total	7.51E+07	41					

Table 39. ANOVA for Center Time in the Open Field Chamber – Measurement #4

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	1.13E+08	7	1.61E+07	3.31	0.00	0.25	0.94
Intercept	3.73E+09	1	3.73E+09	765.15	0.00	0.92	1.00
Stress	8529830.85	1	8529830.85	1.75	0.19	0.02	0.26
Housing	115922.90	1	115922.90	0.02	0.88	0.00	0.05
Sex	2.00E+07	1	2.00E+07	4.10	0.05	0.06	0.52
Stress x Housing	1767235.73	1	1767235.73	0.36	0.55	0.01	0.09
Stress x Sex	7661581.21	1	7661581.21	1.57	0.21	0.02	0.24
Housing x Sex	4.22E+07	1	4.22E+07	8.65	0.00	0.11	0.83
Stress x Housing x Sex	3.05E+07	1	3.05E+07	6.26	0.02	0.08	0.69
Error	3.46E+08	71	4873080.74				

Table 40. ANOVA for Center Time in the Open Field Chamber – Measurement #4 – Males Only

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Stress	12099.313	1	12099.31	0.00	0.96	0.00	0.05
Housing	1.97E+07	1	1.97E+07	4.56	0.04	0.11	0.55
Stress x Housing	9127883.66	1	9127883.66	2.12	0.15	0.05	0.29
Error	1.59E+08	37	4307581.37				
Total	1.88E+09	41					

Table 41. ANOVA for Center Time in the Open Field Chamber – Measurement #4 – Females Only

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Stress	1.56E+07	1	1.56E+07	2.84	0.10	0.08	0.37
Housing	2.25E+07	1	2.25E+07	4.10	0.05	0.11	0.50
Stress x Housing	2.26E+07	1	2.26E+07	4.12	0.05	0.11	0.51
Error	1.87E+08	34	5488477.11				
Total	2.36E+09	38					

Table 42. Descriptive Values for Center Time Ratios (center time/total movement time)

Phase	Open Field 1		Open Field 2		Open Field 3		Open Field 4	
Group	Mean	Std	Mean	Std	Mean	Std	Mean	Std
NSIM	0.59	0.1	1.16	0.1	1.67	0.2	6.27	0.8
NSIF	0.52	0.1	0.95	0.0	1.16	0.1	7.45	0.6
StIM	0.59	0.1	0.97	0.1	1.07	0.1	5.48	1.0
StIF	0.54	0.1	0.89	0.1	1.07	0.1	5.62	0.8
NSSM	0.57	0.1	0.91	0.1	1.00	0.1	5.27	0.8
NSSF	0.60	0.1	0.99	0.1	1.43	0.2	5.00	0.8
StSM	0.73	0.1	1.56	0.6	1.72	0.6	5.44	0.8
StSF	0.57	0.1	0.89	0.1	1.02	0.1	6.36	0.8

Table 43. ANOVA for Center Time Ratios- Measurement #1

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Stress	0.03	1	0.03	0.40	0.53	0.01	0.10
Housing	0.06	1	0.06	0.87	0.36	0.01	0.15
Sex	0.07	1	0.07	1.02	0.32	0.01	0.17
Stress x Housing	0.02	1	0.02	0.22	0.64	0.00	0.07
Stress x Sex	0.04	1	0.04	0.50	0.48	0.01	0.11
Housing x Sex	0.00	1	0.00	0.01	0.92	0.00	0.05
Stress x Housing x Sex	0.06	1	0.06	0.80	0.37	0.01	0.14
Error	5.05	71	0.07				

Table 44. ANOVA for Center Time Ratios- Measurement #1 - Males Only

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Stress	0.20	1	0.20	2.86	0.10	0.07	0.38
Housing	0.07	1	0.07	0.93	0.34	0.02	0.16
Stress x Housing	0.02	1	0.02	0.25	0.62	0.01	0.08
Error	2.64	37	0.07				
Total	13.54	41					

Table 45. ANOVA for Center Time Ratios- Measurement #2

Tests of Between-Subjects Effects

Source	Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Stress	0.12	1	0.12	0.20	0.65	0.00	0.07
Housing	0.18	1	0.18	0.30	0.59	0.00	0.08
Sex	0.94	1	0.94	1.58	0.21	0.02	0.24
Stress x Housing	0.77	1	0.77	1.28	0.26	0.02	0.20
Stress x Sex	0.47	1	0.47	0.79	0.38	0.01	0.14
Housing x Sex	0.12	1	0.12	0.20	0.66	0.00	0.07
Stress x Housing x Sex	0.96	1	0.96	1.60	0.21	0.02	0.24
Error	42.39	71	0.60				

Table 46. ANOVA for Center Time Ratios- Measurement #3

Tests of Between-Subjects Effects

Source	Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Stress	0.17	1	0.17	0.26	0.61	0.00	0.08
Housing	0.05	1	0.05	0.08	0.78	0.00	0.06
Sex	0.77	1	0.77	1.19	0.28	0.02	0.19
Stress x Housing	1.22	1	1.22	1.90	0.17	0.03	0.27
Stress x Sex	0.48	1	0.48	0.75	0.39	0.01	0.14
Housing x Sex	0.06	1	0.06	0.09	0.77	0.00	0.06
Stress x Housing x Sex	3.30	1	3.30	5.12	0.03	0.07	0.61
Error	45.79	71	0.65				

Table 47. ANOVA for Center Time Ratios– Measurement #3 – Males Only

Tests of Between-Subjects Effects

Source	Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Stress	0.15	1	0.15	0.45	0.51	0.01	0.10
Housing	0.26	1	0.26	0.76	0.39	0.02	0.14
Stress x Housing	1.20	1	1.20	3.51	0.07	0.09	0.45
Error	12.70	37	0.34				
Total	61.30	41					

Table 48. ANOVA for Center Time Ratios– Measurement #4

Tests of Between-Subjects Effects

Source	Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Stress	1.51	1	1.51	0.25	0.62	0.00	0.08
Housing	9.14	1	9.14	1.49	0.23	0.02	0.23
Sex	4.65	1	4.65	0.76	0.39	0.01	0.14
Stress x Housing	21.19	1	21.19	3.44	0.07	0.05	0.45
Stress x Sex	0.03	1	0.03	0.01	0.95	0.00	0.05
Housing x Sex	0.51	1	0.51	0.08	0.78	0.00	0.06
Stress x Housing x Sex	6.14	1	6.14	1.00	0.32	0.01	0.17
Error	436.87	71	6.15				

Table 49. ANOVA for Center Time Ratios– Measurement #4 – Females Only

Tests of Between-Subjects Effects

Source	Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Stress	0.54	1	0.54	0.10	0.75	0.00	0.06
Housing	6.73	1	6.73	1.26	0.27	0.04	0.19
Stress x Housing	24.17	1	24.17	4.54	0.04	0.12	0.54
Error	181.19	34	5.33				
Total	1624.71	38					

Table 50. EPM - Descriptive Values for Time in Open Arms (seconds)

	EPM 1		EPM 2	
Group	Mean	Std. Error	Mean	Std. Error
NSlM	14.9	3.90	87.9	11.52
NSlF	29.9	5.89	92.9	7.77
StlM	38.6	7.48	80.6	12.82
StlF	45.4	9.93	116.4	7.77
NSSM	43.2	9.87	62.6	12.10
NSSF	40.1	7.36	103.2	8.80
StSM	31.4	6.99	82.5	10.31
StSF	29.2	7.30	78.9	9.73

Table 51. ANOVA for EPM 1 (Adolescence)– Time in Open Arms

Tests of Between-Subjects Effects

Source	Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Sex	320.91	1	320.91	0.47	0.50	0.01	0.10
Stress	317.06	1	317.06	0.46	0.50	0.01	0.10
Housing	272.51	1	272.51	0.40	0.53	0.01	0.10
Sex x Stress	64.53	1	64.53	0.09	0.76	0.00	0.06
Sex x Housing	876.49	1	876.49	1.27	0.26	0.02	0.20
Stress x Housing	4558.25	1	4558.25	6.60	0.01	0.09	0.72
Sex x Stress x Housing	95.08	1	95.08	0.14	0.71	0.00	0.07
Error	47650.41	69	690.59				
Total	145005.71	77					

Table 52. ANOVA for EPM 1 (Adolescence) Time in Open Arms – Males Only

Tests of Between-Subjects Effects

Source	Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Stress	356.17	1	356.17	0.55	0.46	0.02	0.11
Housing	1134.36	1	1134.36	1.75	0.19	0.05	0.25
Stress x Housing	3184.71	1	3184.71	4.92	0.03	0.12	0.58
Error	23967.82	37	647.78				
Total	71378.37	41					

Table 53. ANOVA for EPM 2 – Time in Open Arms

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Sex	6960.04	1	6960.04	5.18	0.03	0.07	0.61
Stress	161.67	1	161.67	0.12	0.73	0.00	0.06
Housing	2927.36	1	2927.36	2.18	0.15	0.03	0.31
Sex x Stress	207.55	1	207.55	0.15	0.70	0.00	0.07
Sex x Housing	15.61	1	15.61	0.01	0.92	0.00	0.05
Stress x Housing	488.88	1	488.88	0.36	0.55	0.01	0.09
Sex x Stress x Housing	6443.93	1	6443.93	4.79	0.03	0.07	0.58
Error	88713.92	66	1344.15				
Total	692285.50	74					

Table 54. ANOVA for EPM 2
Time in Open Arms – Females Only

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Stress	1.43	1	1.43	0.00	0.97	0.00	0.05
Housing	1685.24	1	1685.24	1.62	0.21	0.05	0.24
Stress x Housing	5241.32	1	5241.32	5.03	0.03	0.13	0.59
Error	34414.15	33	1042.85				
Total	404071.17	37					

Table 55. FST - Descriptive Values for Time Spent Immobile (seconds)

	FST 1		FST 2	
Group	Mean	Std	Mean	Std
NSIM	68.2	15.24	46.2	9.35
NSIF	14.5	4.47	16.5	4.73
StIM	41.2	7.62	29.8	5.25
StIF	6.1	1.44	8.9	1.91
NSSM	76.7	11.43	29.8	5.25
NSSF	20.0	3.35	14.0	3.49
StSM	98.8	14.09	100.5	14.29
StSF	15.0	3.95	15.9	2.89

Table 56. ANOVA for Forced Swim Test – Adolescence

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Sex	58475.06	1	58475.06	56.61	0.00	0.47	1.00
Stress	376.98	1	376.98	0.37	0.55	0.01	0.09
Housing	7177.89	1	7177.89	6.95	0.01	0.10	0.74
Sex x Stress	81.16	1	81.16	0.08	0.78	0.00	0.06
Sex x Housing	2977.66	1	2977.66	2.88	0.09	0.04	0.39
Stress x Housing	3056.18	1	3056.18	2.96	0.09	0.04	0.40
Sex x Stress x Housing	2314.25	1	2314.25	2.24	0.14	0.03	0.31
Error	66104.56	64	1032.88				

Table 57. ANOVA for Forced Swim Test
Adolescence – Males Only
Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Stress	55.57	1	55.57	0.03	0.86	0.00	0.05
Housing	9955.32	1	9955.32	5.34	0.03	0.14	0.61
Stress x Housing	5484.84	1	5484.84	2.94	0.10	0.08	0.38
Error	61481.17	33	1863.07				
Total	274222.95	37					

Table 58. ANOVA for Forced Swim Test
Adolescence – Females Only
Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Stress	393.93	1	393.93	2.64	0.11	0.08	0.35
Housing	443.32	1	443.32	2.97	0.10	0.09	0.39
Stress x Housing	25.11	1	25.11	0.17	0.68	0.01	0.07
Error	4623.39	31	149.14				
Total	12056.42	35					

Table 59. ANOVA for Forced Swim Test – Adulthood
Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Sex	50721.46	1	50721.46	51.12	0.00	0.43	1.00
Stress	59.37	1	59.37	0.06	0.81	0.00	0.06
Housing	15553.49	1	15553.49	15.68	0.00	0.19	0.97
Sex x Stress	23.91	1	23.91	0.02	0.88	0.00	0.05
Sex x Housing	13221.62	1	13221.62	13.33	0.00	0.16	0.95
Stress x Housing	2008.48	1	2008.48	2.02	0.16	0.03	0.29
Sex x Stress x Housing	574.54	1	574.54	0.58	0.45	0.01	0.12
Error	68465.70	69	992.26				

Table 60. ANOVA for Forced Swim Test Adulthood – Males Only

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Stress	4.00	1	4.00	0.00	0.96	0.00	0.05
Housing	29015.07	1	29015.07	15.96	0.00	0.31	0.97
Stress x Housing	2389.39	1	2389.39	1.31	0.26	0.04	0.20
Error	63630.86	35	1818.03				
Total	277618.27	39					

Table 61a. Social Interaction Descriptive Values

(# of interactions with another rat)

SI during Adolescence						
Group	Total Mean	Total Std	Positive Beh Mean	Positive Beh Std	Negative Beh Mean	Negative Beh Std
NSIM	35.6	2.1	4.2	2.1	0.2	0.4
NSIF	33.8	3.3	2.8	1.8	0.1	0.3
StIM	33.9	3.1	3.4	4.6	0.0	0.0
StIF	31.9	3.3	2.8	2.7	0.2	0.4
NSSM	34.1	3.4	3.4	3.3	0.3	0.5
NSSF	32.2	4.0	1.4	1.8	0.3	0.7
StSM	33.6	2.2	3.3	3.2	0.0	0.0
StSF	33.5	2.8	2.8	2.3	0.0	0.0

Table 61b. Social Interaction Descriptive Values
(# of interactions with another rat)

SI during Adulthood						
Group	Total Mean	Total Std	Positive Beh Mean	Positive Beh Std	Negative Beh Mean	Negative Beh Std
NSIM	23.0	4.5	1.4	1.1	0.1	0.3
NSIF	22.8	5.8	1.7	1.7	0.0	0.0
StIM	22.3	3.0	1.8	1.3	0.4	0.9
StIF	23.7	4.4	1.4	1.3	0.8	1.0
NSSM	28.7	3.1	1.6	1.1	0.1	0.3
NSSF	26.1	7.6	1.8	1.8	0.0	0.0
StSM	22.2	4.1	0.5	0.9	0.8	1.0
StSF	25.0	8.5	1.5	1.6	0.6	1.1

Table 62. ANCOVA for Social Interaction Adolescence – Total Behaviors
Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Open Field – Horizontal Activity	58.65	1	58.65	6.94	0.01	0.09	0.74
Sex	16.08	1	16.08	1.90	0.17	0.03	0.28
Stress	23.76	1	23.76	2.81	0.10	0.04	0.38
Housing	6.16	1	6.16	0.73	0.40	0.01	0.13
Sex x Stress	4.84	1	4.84	0.57	0.45	0.01	0.12
Sex x Housing	2.45	1	2.45	0.29	0.59	0.00	0.08
Stress x Housing	24.02	1	24.02	2.84	0.10	0.04	0.38
Sex x Stress x Housing	12.58	1	12.58	1.49	0.23	0.02	0.23
Error	591.67	70	8.45				
Total	89250.00	79					

Table 63. ANCOVA for Social Interaction Adolescence – Total Behaviors – Males Only

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Open Field – Horizontal Activity	3.41	1	3.41	0.48	0.49	0.01	0.10
Stress	24.66	1	24.66	3.48	0.07	0.09	0.44
Housing	3.26	1	3.26	0.46	0.50	0.01	0.10
Stress x Housing	5.22	1	5.22	0.74	0.40	0.02	0.13
Error	255.31	36	7.09				
Total	48309.00	41					

Table 64. ANCOVA for Social Interaction Adolescence – Total Behaviors – Females Only

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Open Field – Horizontal Activity	99.13	1	99.13	11.19	0.00	0.25	0.90
Stress	2.63	1	2.63	0.30	0.59	0.01	0.08
Housing	19.78	1	19.78	2.23	0.15	0.06	0.31
Stress x Housing	60.20	1	60.20	6.79	0.01	0.17	0.72
Error	292.47	33	8.86				
Total	40941.00	38					

Table 65. ANCOVA for Social Interaction Adolescence – Positive Behaviors

Tests of Between-Subjects Effects

Source	Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Open Field – Horizontal Activity	7.93	1	7.93	0.91	0.34	0.01	0.16
Sex	14.01	1	14.01	1.60	0.21	0.02	0.24
Stress	0.09	1	0.09	0.01	0.92	0.00	0.05
Housing	5.68	1	5.68	0.65	0.42	0.01	0.13
Sex x Stress	6.42	1	6.42	0.74	0.39	0.01	0.14
Sex x Housing	1.94	1	1.94	0.22	0.64	0.00	0.08
Stress x Housing	5.46	1	5.46	0.63	0.43	0.01	0.12
Sex x Stress x Housing	2.59	1	2.59	0.30	0.59	0.01	0.08
Error	558.85	64	8.73				
Total	1247.00	73					

Table 66. ANCOVA for Social Interaction Adolescence – Negative Behaviors

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Open Field – Horizontal Activity	0.21	1	0.21	1.47	0.23	0.02	0.22
Sex	0.00	1	0.00	0.03	0.86	0.00	0.05
Stress	0.48	1	0.48	3.37	0.07	0.05	0.44
Housing	0.03	1	0.03	0.21	0.65	0.00	0.07
Sex x Stress	0.05	1	0.05	0.36	0.55	0.01	0.09
Sex x Housing	5.46E-06	1	5.46E-06	0.00	1.00	0.00	0.05
Stress x Housing	0.41	1	0.41	2.86	0.10	0.04	0.38
Sex x Stress x Housing	0.24	1	0.24	1.65	0.20	0.03	0.24
Error	9.18	64	0.14				
Total	12.00	73					

Table 67. ANCOVA for Social Interaction Adolescence – Negative Behaviors – Males Only

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Open Field - Horizontal Activity	0.12	1	0.12	1.38	0.25	0.04	0.21
Stress	0.41	1	0.41	4.72	0.04	0.13	0.56
Housing	0.01	1	0.01	0.16	0.69	0.01	0.07
Stress x Housing	0.01	1	0.01	0.07	0.79	0.00	0.06
Error	2.81	32	0.09				
Total	4.00	37					

Table 68. ANOVA for Social Interaction during Adulthood

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
Horizontal Activity in OF	18.02	1	18.02	0.64	0.43	0.01	0.64	0.12
Sex	8.96	1	8.96	0.32	0.57	0.01	0.32	0.09
Stress	72.65	1	72.65	2.58	0.11	0.04	2.58	0.35
Housing	129.23	1	129.23	4.59	0.04	0.06	4.59	0.56
Sex x Stress	69.86	1	69.86	2.48	0.12	0.03	2.48	0.34
Sex x Housing	6.51	1	6.51	0.23	0.63	0.00	0.23	0.08
Stress x Housing	68.16	1	68.16	2.42	0.12	0.03	2.42	0.34
Sex x Stress x Housing	28.17	1	28.17	1.00	0.32	0.01	1.00	0.17
Error	1970.29	70	28.15					

Table 69. ANCOVA for Social Interaction
Adulthood – Total Social Interaction – Males Only
Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Open Field – Horizontal Activity	12.66	1	12.66	0.94	0.34	0.03	0.16
Stress	148.16	1	148.16	11.02	0.00	0.23	0.90
Housing	94.88	1	94.88	7.05	0.01	0.16	0.73
Stress x Housing	89.03	1	89.03	6.62	0.01	0.16	0.71
Error	484.23	36	13.45				
Total	24344.25	41					

Table 70. ANCOVA for Social Interaction
Adulthood – Positive Behaviors
Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Open Field – Horizontal Activity	1.98	1	1.98	1.09	0.30	0.02	0.18
Sex	3.65	1	3.65	2.00	0.16	0.03	0.29
Stress	3.06	1	3.06	1.68	0.20	0.02	0.25
Housing	0.58	1	0.58	0.32	0.58	0.01	0.09
Sex x Stress	0.23	1	0.23	0.13	0.72	0.00	0.06
Sex x Housing	0.50	1	0.50	0.28	0.60	0.00	0.08
Stress x Housing	2.09	1	2.09	1.15	0.29	0.02	0.19
Sex x Stress x Housing	3.55	1	3.55	1.95	0.17	0.03	0.28
Error	127.38	70	1.82				
Total	297.75	79					

Table 71. ANCOVA for Social Interaction Adulthood – Positive Behaviors – Males Only

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Open Field – Horizontal Activity	3.57	1	3.57	3.37	.08	.09	.43
Stress	2.65	1	2.65	2.50	.12	.07	.34
Housing	.64	1	.64	.60	.44	.02	.12
Stress x Housing	6.97	1	6.97	6.57	.02	.15	.70
Error	38.18	36	1.06				
Total	111.25	41					

Table 72. ANCOVA for Social Interaction Adulthood – Negative Behaviors

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Open Field – Horizontal Activity	0.09	1	0.09	0.18	0.68	0.00	0.07
Sex	0.00	1	0.00	0.00	0.99	0.00	0.05
Stress	6.81	1	6.81	12.78	0.00	0.15	0.94
Housing	0.04	1	0.04	0.08	0.78	0.00	0.06
Sex x Stress	0.28	1	0.28	0.52	0.47	0.01	0.11
Sex x Housing	0.22	1	0.22	0.41	0.53	0.01	0.10
Stress x Housing	0.04	1	0.04	0.08	0.78	0.00	0.06
Sex x Stress x Housing	0.44	1	0.44	0.83	0.37	0.01	0.15
Error	37.32	70	0.53				
Total	55.25	79					

Table 73. ANCOVA for Social Interaction Adulthood – Negative Behaviors – Males Only

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Open Field – Horizontal Activity	0.23	1	0.23	0.42	0.52	0.01	0.10
Stress	2.28	1	2.28	4.16	0.05	0.10	0.51
Housing	0.16	1	0.16	0.30	0.59	0.01	0.08
Stress x Housing	0.51	1	0.51	0.94	0.34	0.03	0.16
Error	19.71	36	0.55				
Total	28.25	41					

Table 74. ANCOVA for Social Interaction Adulthood – Negative Behaviors – Males Only

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Open Field – Horizontal Activity	.01	1	.01	.02	.88	.00	.05
Stress	4.79	1	4.79	9.05	.01	.22	.83
Housing	.08	1	.08	.16	.69	.01	.07
Stress x Housing	.04	1	.04	.07	.79	.00	.06
Error	17.46	33	.53				
Total	27.00	38					

Table 75a. Summary of Biological Findings

	Stress Main Effect			Housing Main Effect			Sex Main Effect	Interaction			Direction of Interaction
Variable	**Overall**	**Males**	**Females**	**Overall**	**Males**	**Females**		**Overall**	**Males**	**Females**	
BW	NS > St	NS > St	----	-----	-----	-----	M > F	Stress x housing^	-----	-----	NSI > NSS > StS > StI
Cort	NS < St	----	-----	-----	-----	-----	M < F				
Chol	----	-----	-----	-----	-----	----	-----	-----	-----	-----	-----
CRP	NS > St	NS > St	NS > St^	I > S	I > S	I > S	-----	Stress x housing	-----	-----	NSI > StI > NSS> StS
Glucose	NS > St	NS > St^	-----	-----	-----	-----	M > F	-----	-----	-----	-----
Insulin	-----	NS > St^	-----	I < S	I < S	-----	M > F	-----	-----	-----	-----
HEART											
Length	*NS > St*	*NS > St*	*NS > St*	*I > S*	*I > S*	*I > S*	-----	-----	-----	-----	-----
Weight	-----	-----	-----	-----	-----	-----	*M > F*	-----	-----	-----	-----
Width	-----	----	-----	*I < S ^*	*I < S ^*	-----	-----	-----	-----	-----	-----
LV	-----	-----	-----	*I < S ^*	*I < S ^*	-----	-----	-----	-----	-----	-----
RV	-----	*NS > St^*	-----	-----	-----	-----	-----	*Stress x housing^*	*Stress x housing^*	-----	*NSS largest StS smallest*
Septal	-----	-----	-----	-----	-----	*I < S*	-----	*Stress x housing^*		*Stress x housing^*	*StS largest NSS smallest*
Lateral	*NS < St*	*NS < St*	-----	-----	-----	-----	*M > F*	-----	-----	-----	-----
Posterior	-----	-----	-----	-----	-----	*I < S^*	-----	-----	-----	-----	-----
Anterior	-----	-----	-----	-----	-----	-----	-----	*Stress x housing^*	-----	-----	*NSS shortest*

^ indicates a trend toward statistical significance $p \leq 0.10$

Table 75b. Summary of Behavioral Findings

	Stress Main Effect			Housing Main Effect			Sex Main Effect	Interaction			Direction of Interaction
Variable	**Overall**	**Males**	**Females**	**Overall**	**Males**	**Females**		**Overall**	**Males**	**Females**	
Food Consumption	-----	NS > St	-----	-----	-----	-----	M > F	Stress x sex	-----	-----	NSM>StM>StF>NSF
OF -Horizontal	-----	-----	-----	----	I < S	-----	M < F	Housing x sex / 3 way	Stress x Housing	Stress x Housing^	M: NSS>StS=St>NSI F:StS=NSI=StI>NSS
OF 1 - Ctr time	-----	-----	-----	----	-----	-----	-----	----	-----	-----	----
OF 2 - Ctr time	-----	-----	-----	----	-----	-----	-----	----	-----	-----	----
OF 3 - Ctr time	-----	-----	-----	----	-----	-----	-----	Stress x Housing^ / 3 way	Stress x housing	-----	M:NSI>StS> NSS=StI
OF 4 - Ctr time	-----	-----	-----	----	I < S	I > S	M < F	Housing x sex / 3 way	-----	Stress x housing	F: NSI>StS>StI>NSS
OF 1 - Ratio	-----	NS > St^	-----	----	-----	-----	-----	----	-----	-----	----
OF 2 - Ratio	-----	-----	-----	----	-----	-----	-----	----	-----	-----	----
OF 3 - Ratio	-----	-----	-----	----	-----	-----	-----	3 way	Stress x Housing^	-----	M: StI>NSS>NSI>StS
OF 4 - Ratio	-----	-----	-----	----	-----	-----	-----	Stress x housing^	-----	Stress x housing	F: NSI>StS>StI>NSS
EPM Adol (in open arms)	-----	-----	-----	----	-----	-----	-----	Stress x housing	Stress x housing	-----	M: NSS>StI>StS>NSI
EPM Adult (in open arms)	-----	-----	-----	----	-----	-----	M < F	3 way	-----	Stress x housing	F: StI>NSS>NSI>StS
FST Adol (time immobile)	-----	-----	-----	I < S	I < S	-----	-----	Sex x housing	NS > St	-----	SM>IM>SF>IF
FST Adult (time immobile)	-----	-----	-----	I < S	I < S	-----	M > F	Sex x housing	NS > St	-----	SM>IM>SF>IF
SI Adol-All Beh	NS > St^	NS > St^	-----	-----	-----	----	-----	-----	-----	Stress x housing	F: NSI>StS>NSS>StI
SI Adol-Neg Beh	NS > St^	NS > St	-----	-----	-----	----	-----	-----	-----	-----	-----
SI Adol-Pos Beh	-----	-----	-----	----	-----	-----	-----	----	-----	-----	----
SI Adult-All Beh	-----	NS > St	-----	I < S	-----	-----	-----	----	Stress x housing	-----	M: NSS>StI=NSI=StS
SI Adult-Neg Beh	NS < St	NS < St	NS < St	----	-----	-----	-----	----	-----	-----	----
SI Adult-Pos Beh	-----	-----	-----	----	-----	-----	-----	Stress x housing	-----	-----	M: NSS=StI=NSI>StS

^ indicates a trend toward statistical significance $p \leq 0.10$

Figures

Figure 1. (in text)

Figure 2. Open Field / Locomotor Chamber

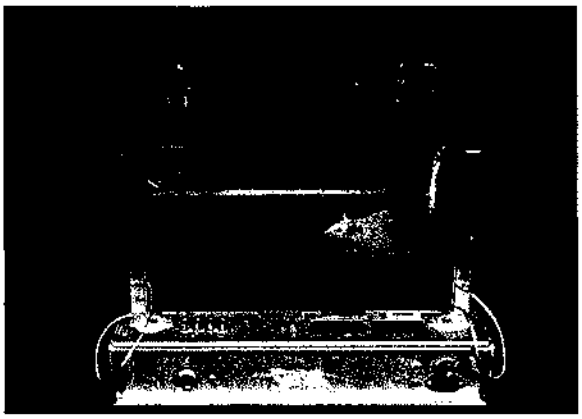

Figure 3. Elevated Plus Maze

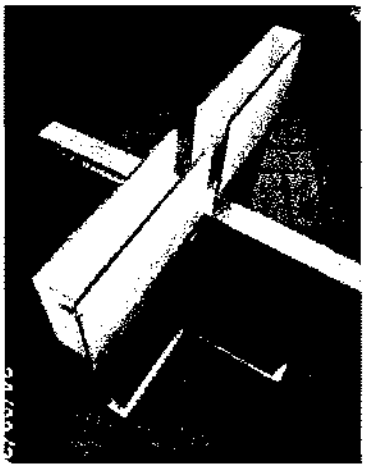

Figure 4. Forced Swim Test

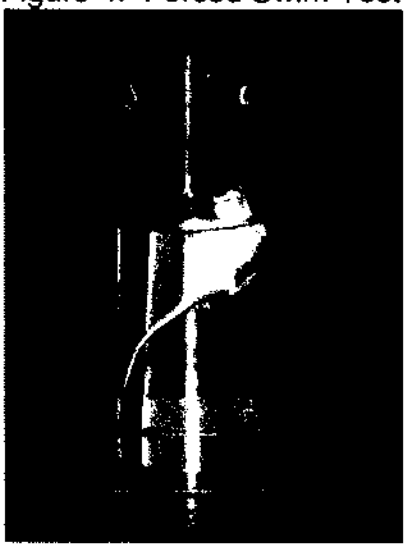

Figure 5. Social Interaction